

Riikka Räisänen

Anthraquinones from the Fungus *Dermocybe sanguinea* as Textile Dyes



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Anthraquinones from the Fungus *Dermocybe sanguinea* as
Textile Dyes

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ABSTRACT

This study is based on the multidisciplinary approach of using natural colorants as textile dyes. The author was interested in both the historical and traditional aspects of natural dyeing as well as the modern industrial applications of the pure natural compounds.

In the study, the anthraquinone compounds were isolated as aglycones from the ectomycorrhizal fungus *Dermocybe sanguinea*. The endogenous β -glucosidase of the fungus was used to catalyse the hydrolysis of the *O*-glycosyl linkage in emodin- and dermocybin-1- β -D-glucopyranosides. The method, in which 10.45 kg of fresh fungi was starting material, yielded two fractions: 56.0 g of Fraction 1 (94% of the total amount of pigment,) consisting almost exclusively of the main pigments emodin and dermocybin, and 3.3 g of Fraction 2 (6%) consisting mainly of the anthraquinone carboxylic acids.

The anthraquinone compounds in Fractions 1 and 2 were separated by one- and two-dimensional thin-layer-chromatography (TLC) using silica plates. 1D TLC showed that neither an acidic nor a basic solvent system alone separated completely all the anthraquinones isolated from *D. sanguinea*, in spite of the variation of the ratios of the solvent components in the systems. Thus, a new 2D TLC technique was developed, applying *n*-pentanol-pyridine-methanol (6:4:3, v/v/v) and toluene-ethyl acetate-ethanol-formic acid (10:8:1:2, v/v/v/v) as eluents. Fifteen different anthraquinone derivatives were completely separated from one another. Emodin, physcion, endocrocin, dermolutein, dermorubin, 5-chlorodermorubin, emodin-1- β -D-glucopyranoside, dermocybin-1- β -D-glucopyranoside and dermocybin, and five new compounds, not earlier identified in *D. sanguinea*, 7-chloroemodin, 5,7-dichloroemodin, 5,7-dichloroendocrocin, 4-hydroxyaustrocorticone and austrocorticone, were separated and identified on the basis of their R_f -values, UV/Vis spectra and mass spectra. One substance remained unidentified, because of its very low concentration.

The anthraquinones in Fractions 1 and 2 were preparatively separated by liquid-liquid partition, with isopropylmethyl ketone and aqueous phosphate buffer as the solvent system. Advantage was taken of the principle of stepwise pH-gradient elution. The multiple liquid-liquid partition (MLLP) offered an excellent method for the preparative separation of compounds, which contain acidic groups such as the phenolic OH and COOH groups. Due to their strong aggregation properties, these compounds

are, without derivatization, very difficult to separate on a preparative scale by chromatographic methods. By the MLLP method remarkable separations were achieved for the components in each mixture. Emodin and dermocybin were both obtained from Fraction 1 in a purity of at least 99%.

Pure emodin and dermocybin were applied as mordant dyes to wool and polyamide and as disperse dyes to polyester and polyamide, using the high temperature (HT) technique. A mixture of dermorubin and 5-chlorodermorubin was applied as an acid dye to wool. In these experiments, synthetic dyes were used as references. Experiments were also performed using water extract of the air-dried fungi as dye liquor for wool and silk. The main colouring compounds in the crude water extract were emodin and dermocybin, which indicated that the *O*-glycosyl linkages in emodin- and dermocybin-1- β -D-glucopyranosides were broken by the β -glucosidase enzyme. Apparently, the hydrolysis occurred during the drying of the fungi and during the soaking of the dried fruit bodies overnight when preparing the dyebath. The colour of each dyed material was investigated in terms of the CIELAB L^* , a^* and b^* values, and the colour fastness to light, washing and rubbing was tested according to the ISO standards.

In the mordant dyeing experiments, emodin dyed wool and polyamide yellow and red, depending on the pH of the dyebath. Dermocybin gave purple and violet colours. The colour fastness of the mordant-dyed fabrics varied from good to moderate. The fastness properties of the natural anthraquinone carboxylic acids on wool were good, indicating the strength of the ionic bonds between the COO^- groups of the dyes and the NH_3^+ groups of the fibres. In the disperse dyeing experiments, emodin dyed polyester bright yellow and dermocybin bright reddish-orange, and the fabrics showed excellent colour fastness. In contrast, emodin and dermocybin successfully dyed polyamide brownish-orange and wine-red, respectively, but with only moderate fastness. In industrial dyeing processes, natural anthraquinone aglycone mixtures dyed wool and silk well even at low concentrations of mordants, *i.e.* with 10% of the weight of the fibre (owf) of $\text{KAl}(\text{SO}_4)_2$ and 1 or 0.5% owf of other mordants.

This study showed that purified natural anthraquinone compounds can produce bright hues with good colour-fastness properties in different textile materials. Natural anthraquinones have a significant potential for new dyeing techniques and will provide useful alternatives to synthetic dyes.

Veriseitikin (*Dermocybe sanguinea*) antrakininonit tekstiiliväreinä

TIIVISTELMÄ

Tutkimuksessa on haluttu lähestyä luonnon väriaineilla värjäämistä monitieteellisestä näkökulmasta. Kirjoittaja on ollut kiinnostunut luonnon väriaineiden historiasta ja perinteisistä värjäysmenetelmistä sekä puhtaiden luonnon väriaineiden uusista teollisen värjäyksen sovellutuksista.

Antrakininonit eristettiin veriseitikistä uudella menetelmällä, jossa käytettiin hyväksi sienen omaa β -glukosidaasi entsyymiä. Entsyymi katalysoi emodiini- ja dermosybiini-1- β -D-glukosidien O-glykosyylisidoksen hydrolyysiä. Eristysmenetelmällä, jossa lähtöaineena oli 10,45 kg tuoreita sieniä, saatiin kaksi fraktiota: 56,0 g Fraktiota 1 (94 % kokonaispigmenttimäärästä) ja 3,3 g Fraktiota 2 (6 %). Fraktio 1 sisälsi pääosin emodiinia ja dermosybiiniä kun taas Fraktio 2 sisälsi enimmäkseen antrakininonikarboksyylihappoja.

Eristettyjen antrakininonyhdisteiden analysointiin kehitettiin yksi- ja kaksi-dimensionaaliset TLC-menetelmät. Yksi-dimensionaalinen TLC menetelmä osoitti ettei happamalla eikä emäksisellä liuotinsysteemillä pystytty erottamaan kaikkia seoksessa olevia antrakininonyhdisteitä toisistaan. Siksi kehitettiin kaksi-dimensionaalinen TLC menetelmä, jossa *n*-pentanoli-pyridiini-metanoli (6:4:3, v/v/v) -seos ja tolueeni-etyyliasetatti-etanoli-muurahaishappo (10:8:1:2, v/v/v/v) -seos toimivat eluenteina. Kaksi-dimensionaalisella menetelmällä pystyttiin erottamaan viisitoista antrakininonyhdistettä toisistaan. Emodiinin, fyskionin, endokrosiinin, dermoluteiinin, dermorubiinin, 5-klooridermorubiinin, emodiini-1- β -D-glukopyranosidin, dermosybiini-1- β -D-glukopyranosidin and dermosybiinin lisäksi löydettiin viisi aikaisemmin tässä sienessä tunnistamatonta antrakininonyhdistettä. Nämä olivat 7-klooriemodiini, 5,7-diklooriemodiini, 5,7-diklooriendokrosiini, 4-hydroksiaustrokortikoni ja austrokortikoni. Yhdisteet karakterisoitiin R_f -arvojen sekä UV/Vis ja massaspektrien avulla. Yksi yhdiste jäi identifioimatta erittäin vähäisen ainemäärän vuoksi.

Pigmenttien preparatiivinen eristäminen antrakininonseoksista Fraktioista 1 ja 2 suoritettiin multippeli neste-neste-partitiolla (MNNP) käyttämällä isopropyylimetyyliketonia ja fosfaattipuskuria liuotinsysteeminä. Separoinnissa käytettiin hyväksi puskuriliuoksen asteittaista pH-gradienttia. MNNP sopi erittäin hyvin separointimenetelmäksi yhdisteille, jotka sisälsivät happamia ryhmiä, kuten fenolisia OH-ryhmiä ja COOH-ryhmiä. Voimakkaan aggregaatiotaipumuksensa vuoksi tällaiset yhdisteet on ilman

derivatisointia vaikea separoida preparatiivisessa mittakaavaassa kromatografisilla menetelmillä. MNNP:lla saavutettiin hyvä komponenttien erottuminen molemmissa antrakinoniseoksissa. Emodiinin ja dermosybiinin puhtausasteiksi määritettiin 99 %.

Puhdasta emodiinia ja dermosybiiniä käytettiin peittaväriaineina villalle ja polyamidille sekä dispersio-väriaineina polyesterille ja polyamidille. Dermorubiinin ja 5-klooridermorubiinin seosta kokeiltiin happovärinä villalle. Puhtailla luonnon yhdisteillä saatujen värjäysten tuloksia verrattiin synteettisillä väriaineilla saatuihin tuloksiin. Villaa ja silkkiä värjättiin myös kuivatuista sienistä eristetyillä väriliemillä. Emodiini ja dermosybiini olivat vesiuutteen pääasialliset väriaineet. Tästä voidaan päätellä, että emodiini- ja dermosybiini-1- β -D-glukopyranosidien O-glykosyylisidokset olivat katkenneet β -glukosidaasi entsyymien toimesta sienten kuivausprosessin aikana sekä edelleen liotuksen aikana, kun kuivatuista sienistä valmistettiin värjäysliuosta. Värjättyjen tekstiilien väri määritettiin CIELAB-järjestelmän mukaisina L*, a* ja b* arvoina. Värjättyjen tekstiilien laatua testattiin suorittamalla ISO standardien mukaiset värien valon, hankauksen ja vesipesun kestokokeet.

Peittävärjäyskokeissa emodiini värjäsi villan ja polyamidin keltaiseksi tai punaiseksi riipuen siitä oliko värjäysliemen pH noin 4 vai lähellä neutraalia. Dermosybiinillä saatiin peittävärjäyskokeissa sinertäviä ja violetteja värejä. Peittävärjättyjen tekstiilien värinkestot vaihtelivat kohtalaisesta hyvään. Antrakinonikarboksyylihappoilla värjätyn villan värinkestot, erityisesti pesun kestot, olivat hyvät, mikä kertoo väriaineen COO⁻-ryhmän ja kuidun NH₃⁺-ryhmän välisen ionisidoksen voimakkuudesta. Dispersiivärjäysmekanismilla emodiini värjäsi polyesterin kirkkaan keltaiseksi ja dermosybiini kirkkaan oranssiksi. Kankaiden värinkestot olivat erinomaisia. Polyamidin värjäys emodiini- ja dermosybiini-dispersiiväreillä onnistui hyvin, mutta näytteiden värinkestot olivat ainoastaan kohtalaiset. Kokeet luonnon antrakinoniseoksilla osoittivat, että perinteistä kasvivärjäystä on mahdollista suorittaa käyttämällä hyväksi värjäyskoneita. Kohtuulliset ja joissakin tapauksissa jopa hyvät värinkestot saatiin aikaan kokeissa, joissa alunaa käytettiin 10 % ja muita peitta-aineita vain 0.5–1.0 % värjättävän materiaalin painosta.

Tämä tutkimus osoitti, että puhtaista luonnon väriaineista voidaan saada aikaan kirkkaita värejä, joilla on hyvät värinkesto-ominaisuudet erilaisissa tekstiilimateriaaleissa. Luonnon antrakinonit soveltuvat useiden eri materiaalien värjäämiseen eri tekniikoilla ja ne tarjoavat tulevaisuudessa varteenotettavan vaihtoehdon synteettisille väriaineille.

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	PUBLICATIONS I–VI	

ABBREVIATIONS

CA	cellulose acetate
CC	column chromatography
CI	Colour Index
CIE	Commission Internationale de l'Eclairage
CO	cotton
CTA	cellulose triacetate
1D	one-dimensional
2D	two-dimensional
EtOH	ethanol
EU	European Union
GC	gas chromatography
HPLC	high performance liquid chromatography
HT	high temperature
IMK	isopropylmethyl ketone
ISO	International Organization for Standardization
LC	liquid chromatography
LC ₅₀	lethal concentration which leads to the death of 50% of the test animals
MeOH	methanol
MLLP	multiple liquid-liquid partition
MO	molecular orbital
MS	mass spectrometry
owf	of the weight of the fibre
PA	polyamide
PES	polyester
SE	silk
SKY	Suomen Käsityön Ystävät (The Friends of Finnish Handicraft)
SSF	solid state fermentation
TLC	thin layer chromatography
UV	ultra violet
VB	valence bond
Vis	visible light
WO	wool
v/v	volume/volume

1 INTRODUCTION AND LIST OF ORIGINAL PUBLICATIONS

Dyeing with pigments from plants, roots, barks, lichens *etc.* is part of the old Finnish craft tradition, the restoration and maintenance of which are important. This has significance in keeping craft culture alive as well as in utilizing the characteristic craft tradition in entrepreneurship. However, to ensure the high quality of the products, more research on natural dyeing is required, for example, into the uniformity of the colour and the high colour fastness of the materials. Research is also needed for adapting the old dyeing traditions and recipes to current conditions, which must take into account the environmental aspects. In the author's previous thesis for a master's degree,¹ the amounts of mordants used in the numerous home-dyeing books were stated to be excessive. In that thesis, the effect of various amounts of alum and CuSO_4 on colour hue and colour fastness in woollen yarn was studied. Wool was dyed with the pigments extracted with water from the green leaves of birch trees. In green leaves, the main colouring components suitable for dyeing, *i.e.* being soluble in water, were xanthophylls, which effected the yellow colour of the dyed wool yarn.^{2,3} Flavonoids are also water soluble and they might have been present in the dye liquor as minor compounds. The green colour of plants is produced by chlorophylls, which, however, decompose easily and thus are not possible for dyeing. According to the results of the thesis,¹ amounts of alum exceeding 15% owf (of the weight of the fibre) did not change the hue or improve the colour fastness in the arranged test set-up. For CuSO_4 , the corresponding amount was observed to be 10% owf. According to the study of Nicolai and Nechwatal, an amount of 10% owf of alum mordant is adequate.⁴ However, it seems that the results of research reach the home and craft dyers very slowly. Even in some of the newest domestic dyeing recipes the amounts of alum vary from 10–20%, being even 25% owf, and the amounts of CuSO_4 seem to vary from 5–10% owf.^{5,6} These amounts are considered to be excessive and reduction of the amounts of chemicals to the minima is highly desirable. However, more research is required on the optimisation of the dyeing conditions.

Natural dyeing using the colorants of plants and fungi is quite common in Finland.^{7,8} However, published reports of scientific research on the subject are very rare. The International Fungi and Fibre Symposium was held in Rovaniemi, Finland, in August 2001. The agenda of the meeting, among other things, listed the lack of experts in the field of fungi and natural dyeing as a problem. It seems that home and craft dyers

are willing to develop their dyeing methods, if information on the subject is available. The basis for the improvements in dyeing techniques is research results, which, in addition, lead to better quality of the dyed products.

The current study is significant not only for the craft culture but also for the technical and economic applications of natural raw materials in industry. As a renewable source of raw material, plants and fungi could be utilized more widely as a source of pigments. Furthermore, in the future, natural pigments could be isolated from Nature, using advanced techniques. Natural dyes offer an alternative for sparing fossil resources, particularly when enzyme technology combined with genetic engineering or cell culture is used to its full potential. It is known that the total syntheses of substituted anthraquinones are relatively complicated. Frequently, toxic reagents, such as mercury salts or chromic acid, are used in the reactions. In addition, toxic side products may be produced. In a large-scale production, purification of dyestuffs after synthesis is often difficult and expensive. To avoid these disadvantages, it should be noted that Nature is often the best synthesizer. In the biosynthetic processes, toxic side products or wastes are not produced. It would be optimal to isolate the pigments, *e.g.* anthraquinones, from the Nature by simple techniques and, if required, modify them further by chemical means.

By combining the research traditions of the two branches of science, organic chemistry and craft science, a more profound understanding of the dyeing textiles may be obtained. A knowledge of chemistry has been necessary for understanding the character of the dyes as well as the whole process of dyeing. Natural dyeing, belonging to the craft culture, forms part of the research field of craft science. Because this research was a pilot study on the use of pure natural compounds as dyes for textiles, it fulfils the main purpose of the universities, which is to perform scientific research. Thus, its place in craft science and its place in chemistry are both justifiable. In this study the author has been interested in both the traditional and the industrial applications of natural anthraquinone pigments. The dyeing experiments with the anthraquinone aglycones in the crude water extract of the air-dried fungi represented traditional dyeing, whereas the experiments with the pure natural anthraquinone aglycones represented new applications. This thesis is based on the original publications, which are referred to in the text by their Roman numerals (I–VI). The author's contribution to the research represented in the publications is explained in Appendix 1.

In colour chemistry, colorants are considered either dyes or pigments: "*Dyes* are applied to various substrates, for example textile, paper, *etc.*, from a liquid in which they are completely, or at least partly, soluble. Dyes possess a specific affinity to the substrates for which they are used. *Pigments* are insoluble in the media in which they are applied and they have to be attached to substrates by additional compounds, *e.g.* by a polymer in a paint".⁹ However, this study is about natural compounds and it covers several areas of science, which define same words differently. For example, in biology the term "pigment" is used in a different meaning than in the colour chemistry. Thus, in this study terms colorant and pigment are used as synonyms, and when a pigment or a colorant is used in dyeing purposes the term dye may be used.

List of original publications

- I P. H. Hynninen, R. Räisänen, P. Elovaara and E. Nokelainen, Preparative isolation of anthraquinones from the fungus *Dermocybe sanguinea* using enzymatic hydrolysis by the endogenous β -glucosidase, *Z. Naturforsch.* **2000**, 55c, 600–610.
- II R. Räisänen, H. Björk and P. H. Hynninen, Two-dimensional TLC separation and mass spectrometric identification of anthraquinones isolated from the fungus *Dermocybe sanguinea*, *Z. Naturforsch.* **2000**, 55c, 195–202.
- III P. H. Hynninen and R. Räisänen, Stepwise pH-gradient elution for the preparative separation of natural anthraquinones by multiple liquid-liquid partition, *Z. Naturforsch.* **2001**, 56c, 719–725.
- IV R. Räisänen, P. Nousiainen and P. H. Hynninen, Emodin and dermocybin natural anthraquinones as mordant dyes for wool and polyamide, *Textile Res. J.* **2001**, 71 (11), 1016–1022.
- V R. Räisänen, P. Nousiainen and P. H. Hynninen, Dermorubin and 5-chlorodermorubin natural anthraquinone carboxylic acids as dyes for wool, *Textile Res. J.*, **2002**, 72 (11), 973–976.
- VI R. Räisänen, P. Nousiainen and P. H. Hynninen, Emodin and dermocybin natural anthraquinones as high-temperature disperse dyes for polyester and polyamide, *Textile Res. J.* **2001**, 71 (10), 922–927.

2 DYES AND COLOUR

The first evidence of man using natural colours lies in the cave paintings in the north of Spain and the south of France from the era 15000–9000 BC. Coloured pigments were produced from stones containing iron, manganese and aluminium by burning them with plant or animal material.^{10,11} In primitive cultures, human bodies were coloured with paints as if the bodies were clothed. Elsewhere, natural tones of wool and cotton were mixed together in variable amounts to obtain different hues. Textiles were also coloured by painting the material with mineral pigments, using saliva, oil, wax or egg white as a binder. The art of dyeing was known quite early. The Egyptian hieroglyphs show that mordant dyeing with aluminium was among the first techniques used, even though aluminium mordant itself was not yet known.^{9,10}

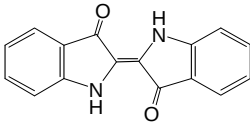
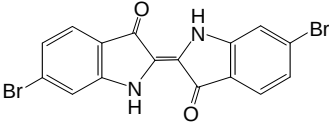
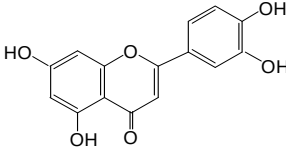
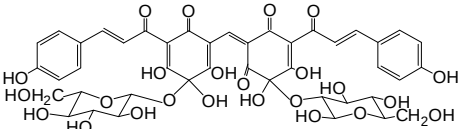
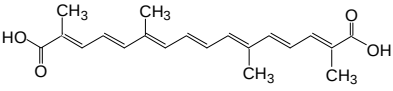
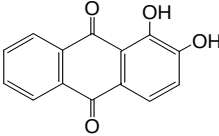
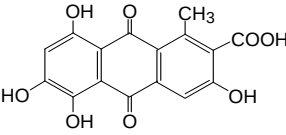
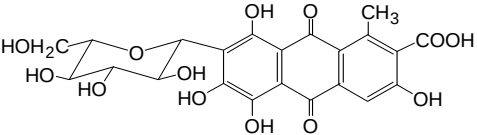
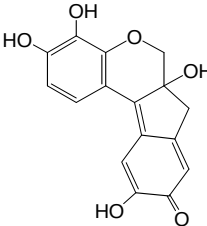
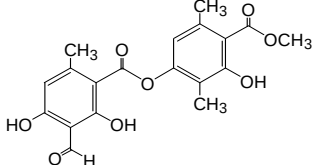
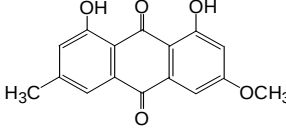
2.1 Natural dyes

The problem in tracing historical dyed textiles is that both the materials and the natural pigments easily decompose and samples are often contaminated with dirt. The first evidence of pieces of dyed textiles date back to *ca.* 3000 BC in Egypt. In the classical world, bright shades of red, blue and yellow were produced, using pigments from several sources of plants, insects and molluscs. Green colour was usually obtained by dyeing the piece of cloth twice: firstly in a dyebath giving a yellow tone and secondly in a dyebath giving a blue tone or *vice versa*.^{12,13}

The principle blue dye used was indigo (Table I), the main sources of which were woad (*Isatis tinctoria* L.) and the indigo plant (*Indigofera tinctoria* L.).^{14,15} The indigo plant grows in the tropics and woad in the temperate zones.¹⁰ Of the several sources of yellow dyes, the most important were weld from *Reseda luteola* L., safflower from *Carthamus tinctorius*, and saffron from *Crocus sativus* (Table I).^{15,16} In Northern Europe, yellow was also obtained from ling (*Calluna vulgaris*) and clubmoss (*Lycopodium* species).^{17,18}

Red colour was most commonly obtained from the hairy roots of madder (*Rubiaceae* sp.), whose main colouring component is alizarin (Table I).^{12,19} Madder was used for the preparation of the famous Turkey red – a colour, which required a series of different operations. The structure of Turkey red is not yet fully determined, but

Table I. Chemical structures of the main colouring components of some historical natural dyes.^{15,16}

Dye source	Latin name	The main colouring component and its chemical structure	
Indigo plant	<i>Indigofera tinctoria</i>	indigo	
Woad	<i>Isatis tinctoria</i>	indigo	
Mollusc	<i>Hexaplex trunculus</i>	indigo and 6,6'-dibromoindigo	
Weld	<i>Reseda luteola</i>	luteolin	
Safflower	<i>Carthamus tinctorius</i>	carthamin	
Saffron	<i>Crocus sativus</i>	crocin	
Madder	<i>Rubiaceae</i> sp.	alizarin	
Kermes	<i>Kermes vermilio</i>	kermesic acid	
Cochineal	<i>Dactylopius</i> sp.	carminic acid	
Logwood	<i>Haematoxylum campechianum</i>	haematein	
Lichen	<i>Parmelia</i> sp.	atranorin	
	<i>Xanthoria</i> sp.	physcion	

it is proposed to be a calcium-aluminium-alizarinate, a 1:2 complex of aluminium with alizarin.^{20,21} The most prestigious red dyestuff was obtained from the insect *Kermes vermilio*, which lives on the kermes oak (*Quercus coccifera*) in the Middle East.^{12,22,23} There are also types of kermes that live on the roots of certain plants, for example the Polish kermes, which live on the root system of the perennial host plant *Scleranthus perennis*. The Polish kermes is grown in Middle and Eastern Europe.²² The main colorant in *Kermes vermilio* is kermesic acid (Table I) whereas in Polish kermes it is carminic acid, and because of this, Polish kermes is also called Polish cochineal. Kermes and madder were the red dyes known in the Old World before the discovery of America in the 16th century.¹² The cochineal insect of the family *Dactylopius* lives in a cactus. The insect is native to Central and South America, where the Aztecs had extracted the dye from the insect centuries before the coming of the Spaniards. The colorant, the main component of which is carminic acid, is obtained from the female insect (Table I). Cochineal was transplanted to the South of Europe and the Canary Islands after the discovery of America.^{12,22,24,25} When the production of cochineal in Europe began, it soon replaced the imported kermes, and the use of madder was also reduced.²⁶

Methods of mixing red and blue colours to produce purple were widely used in classical times.¹² The blue component was indigo (Table I) and the red was obtained from madder or kermes.¹² However, a dyestuff that gave purple itself was obtained from the glands of molluscs of several types. The colour called Tyrian purple was obtained from a shellfish of the species *Murex trunculus* (= *Hexaplex trunculus*).^{12,27,28} As many as 10 000 molluscs were needed to produce 1 g of dyestuff. The dye was very expensive and so could be afforded only by socio-politically and religiously significant people.²⁸⁻³¹

In Middle and Northern Europe, red and purple colours were also obtained from lichen.^{12,14,17,32-39} The Vikings knew about lichen dyeing and they apparently spread the tradition.^{34,35,40} The use of lichen for textile dyeing was relatively common and the tradition has survived until today, as evidenced by the lichen dyeing recipes in numerous books on home-dyeing.^{5,6,18,41-43}

Black colour was obtained from logwood (Table I), which is among the few natural colorants still used in industry. Nowadays, logwood is used to dye silk and leather.⁴⁴

The fastness of indigo and logwood is exceptionally high for natural dyes. From the earliest of times, mordants were used not only to change the colour, but also to

improve its fastness.⁴⁵ The light fastness of chrome-mordanted textiles is higher than that of textiles mordanted with alum or copper, iron and tin salts. However, the use of chrome as a mordant is a relatively modern technique, which has been used from the early 18th century onwards.⁴⁶

There is very little information about the use of fungi for dyeing textiles. In his book on dyeing, the Swede Johan Linder mentions old *Lactarius* (milk cap) as a source of yellow colour.⁴⁷ In Europe, *Pisolithus tinctorius* was among the first fungi to be used in dyeing and the word *tinctorius* refers to its colouring nature. It is known that American Indians used a red pigment obtained from the fungus *Echinodontium tinctorium* for their war paint. Moreover, in America furs were sometimes coloured using extracts of fungi to give them a luxurious golden hue. In the East, the Chinese produced red colorant from *Monascus purpureus*, but for what purpose they used it is not clear. During the Second World War, some experiments were carried out in Europe, aiming at the use of fungi as a source of textile colorants, but it was not until the 1970's that dyeing with fungi became popular among craft workers.^{48,49} In 1974 the American Miriam C. Rice published the first book concerning dyeing with fungi and, since then, the custom has spread all over the world.^{6,50–55}

2.2 Synthetic dyes

The economic limitations of natural dyes became apparent in the late 18th century, when the industrial revolution began in Europe. The first attempts to synthesize dyestuffs were made in 1771, when Woulfe began his investigation on indigo and discovered picric acid, which dyed silk greenish yellow.^{9,44,56} However, picric acid remained insignificant as a textile dye because of its poor light-fastness.⁵⁶ The development of synthetic dyes started in the middle of the 19th century. In 1856 the 18-year-old chemistry student William Henry Perkin discovered a brilliant purple solution while attempting to synthesize quinine. Perkin immersed a piece of silk fabric in the solution and observed that the colour of the fabric did not wash out or fade upon exposure to sunlight.^{9,57–60} A year later he left the Royal College of Chemistry in London and set up the first dye factory in the world, which produced the first synthetic dye he had discovered, Mauveine (Colour Fig. 1 in Appendix 2).^{9,57–60}

Though Perkin's success with Mauveine had a stimulating effect on the search for new synthetic dyes, it was also the fundamental work by Kekulé on the quadrivalence of carbon (1858) and on the constitution of benzene (1865) which made possible the planned preparation of synthetic dyes and the artificial production of natural dyes.^{9,61} In 1868, three years after Kekulé had discovered the structure of benzene, Graebe and Liebermann elucidated the structure of alizarin. Within a few years, synthetic alizarin had displaced the natural product from the market place.^{9,61} Elucidation of the structure and synthetic pathway for indigo involved research over several decades.⁹

Within fifty years from the discovery of the first synthetic dye, such products accounted for over 90% of the dyes used.⁵⁷ The fastness of the first synthetic colorants was poor compared with the natural ones used at the time. In the beginning, however, the bright shades of the new dyes were so fascinating that little interest was paid to their properties. By the year 1905, nearly every imaginable colour had been formed. As the number of shades increased, more research was needed to find new colorants. Thus, scientists concentrated on discovering faster, more easily applicable and cheaper dyes.⁶⁰

The development of man-made fibres, *e.g.* polyamide, polyester and polyacrylonitrile, in the 20th century presented new challenges to the dyestuff industry. Though specific dye types had to be used for these fibres, the new colorants were mere modifications of available ones.^{58,62} Costs of oil and energy as well as the strict toxicological and environmental constraints have directed recent trends in the dye industry. Efforts have been devoted to developing better and cheaper pathways for existing dyes.^{62,63}

2.2.1 Classification of dyes

Dyes may be classified in several ways. They may be arranged according to their chemical structure (for example azo, anthraquinone and sulfur dyes), or fibre type (dyes for nylon, cotton, wool *etc.*) or method of application (such as acid, mordant and reactive dyes). The overlap of these classifications is obvious, because there is hardly a chemical class of dye which occurs solely in one group and *vice versa*. Furthermore, some chemical groups can be applied to two or more substrates.^{64–66}

The Society of Dyers and Colourists in Great Britain and the American Association of Textile Chemists and Colourists in the USA jointly publish the Colour Index (CI), which is the internationally accepted catalogue of dyes and pigments (For a definition of a dye and a pigment, see p. 11). The first edition of the CI was published in 1924. The CI lists all the dyes and pigments used commercially for large-scale dyeing. The classification is based on the method of application and the chemical constitution. In the CI, each dye or pigment is given two reference numbers, one for the colouristic and the other for the chemical classification. For alizarin, for example, CI Mordant Red 11 is the so-called “CI Generic Name”, which refers to the colouring properties, whereas CI 58000 identifies the chemical structure.^{65–67}

2.2.2 Toxicity of synthetic dyes

The wide diversity of the chemical structure of organic colorants carries with it a fairly broad spectrum of toxicological properties.⁶⁸ Generally, dyes exhibit low toxicity to mammals and aquatic organisms. In tests on fish, only 2% of dyes had an LC_{50} of under 1.0 mg/l and over 96% of dyes had an LC_{50} above 10 mg/l.^{68,69} In a survey of about 4500 dye products, when the dyes were fed to rats, 1% of the textile colorants obtained an LC_{50} under 250 mg/kg, and 82% an LC_{50} above 5000 mg/kg.^{68,70}

Most of the toxic dyes are found among the diazo and cationic dyes.^{70,71} The first indication that aromatic amines might exert carcinogenic effects appeared in 1895, when bladder cancer was described among employees in the manufacture of the dye fuchsin.⁷² A great number of diverse aromatic amines have now been studied for carcinogenicity and some of these dyes have been abandoned because of their carcinogenic risk.⁷² However, aromatic amines are a very important group of industrial chemicals and, for example, azo dyes are still one of the most widely used class of dyestuffs in the textile industry.

In fairly rare cases, dyes cause acute toxicity. More common, however, are the irritations and allergies that they may cause. Some azo and anthraquinone dyes are found to cause tactile allergies.⁷⁰ In addition to assessing the toxicity of dye components, it is important to determine the overall toxicity of the various chemicals in a fabric. The toxicity of the fabric cannot be predicted directly from the toxicity of the

dye itself, because other chemicals may alter the cytotoxicity, for example the ones in the flame-retardant as well as the water- and soil-repellent finishes.⁷³

2.2.3 Colour

Colour and colour vision are the manifold phenomena of physical, chemical, physiological and psychological processes. Of the electromagnetic spectrum, only the wavelengths in the range between 400 and 700 nm are visible to man. The appearance of a coloured object is a process depending on selective absorption of certain wavelengths of visible light combined with reflection, scattering and transmission of the non-absorbed radiation (Fig. 1).⁷⁴⁻⁷⁶ When the reflected, scattered or transmitted light reaches the retina of the human eye, it is absorbed by photoreceptors and converted into a complex sequence of neural reactions. In the brain, the sensory input is processed and evaluated, leading to visual perception.^{74,77,78}

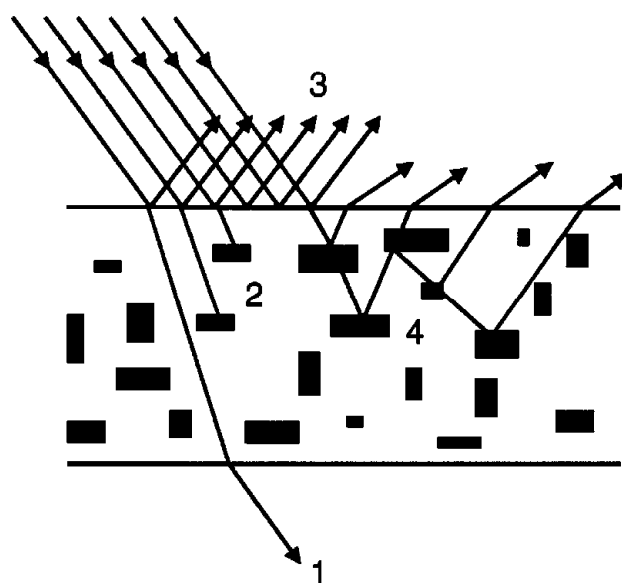


Figure 1. Light interactions within a coloured surface: transmission (1), absorption (2), reflection (3) and scattering (4). The figure has been modified by the author.^{75,76} (Reproduced with permission of the copyright owner, © 1989 Society of Dyers and Colourists).

One of the problems in defining colour is the subjective character of the colour phenomenon. The appearance of an object is dependent on many factors, such as illumination, background and surrounding colours as well as the size and nature of the

object. Moreover, the observer exerts an influence as he interprets the colours according to his experience.⁷⁹ Ever since Aristotle in 350 BC, there have been numerous attempts to compile colour-order systems for defining colour.⁸⁰ Munsell made one of the most famous discoveries of the three-dimensional model, when he described colour in terms of hue, value and chroma.⁸¹ His solid colour atlas contained a series of charts, each of which corresponded to a particular hue. The atlas was published in 1915 and ever since has been the standard most widely used. The principle of the Munsell system for defining colour is based on visual perception and comparison between the colour of the sample and the colour of a standard chip.^{80–82}

Colorimetry is a term used for the quantitative description of colours as they appear to the human eye.^{74,82} In 1931, the CIE (Commission International de l'Eclairage) established a system for the physical measurement of colour.⁸³ In the CIE system, the light reflected from the sample surface is recorded, using a colour-measurement spectrophotometer.⁸⁴ The spectrophotometric measurement is made relative to a reference standard which has a reflectance of unity at all points across the spectrum. The reference standard is usually a white ceramic tile or an opal glass plate.⁸⁴ The CIE system for numerical specification of colour is based on the definition that light reflected from a coloured surface can be visually matched by an additive mixture of red, green and blue light in suitable proportions.⁸⁵

The CIE has defined the standard conditions for colour determination; these are a standard observer, standard illuminants, standard illumination and viewing geometries and a particular set of primaries [X], [Y] and [Z].⁷⁹ The tristimulus values of a particular colour are the amounts of X, Y and Z that are derived from the reflectance spectrum of the colour. X represents the amount of red, Y the amount of green and Z the amount of blue primary required to obtain the colour. Y is also a direct measure of luminosity.^{79,82,85} To relate the tristimulus values of the sample to its real colour appearance, some defining has to be made. The X, Y and Z are converted into so-called chromaticity co-ordinates x, y and z, where $x = X/(X + Y + Z)$, $y = Y/(X + Y + Z)$ and $z = Z/(X + Y + Z)$. In the CIE chromaticity diagram (Fig. 2), x is the abscissa and y the ordinate. The spectral colours lie on a parabolic curve.^{79,85,86} Colours with x and y values close to the locus of the spectrum are very saturated colours with hues close to those of the corresponding spectral colours.⁷⁹ Although the CIE system provided an unambiguous description of colours, there was serious non-uniformity in a colour-difference formula. The CIE system was not able to define tolerances among samples

differing in hue, which led, for example, to problems in the determination of colorimetric pass and fail limits in colour matching.^{83,85,87}

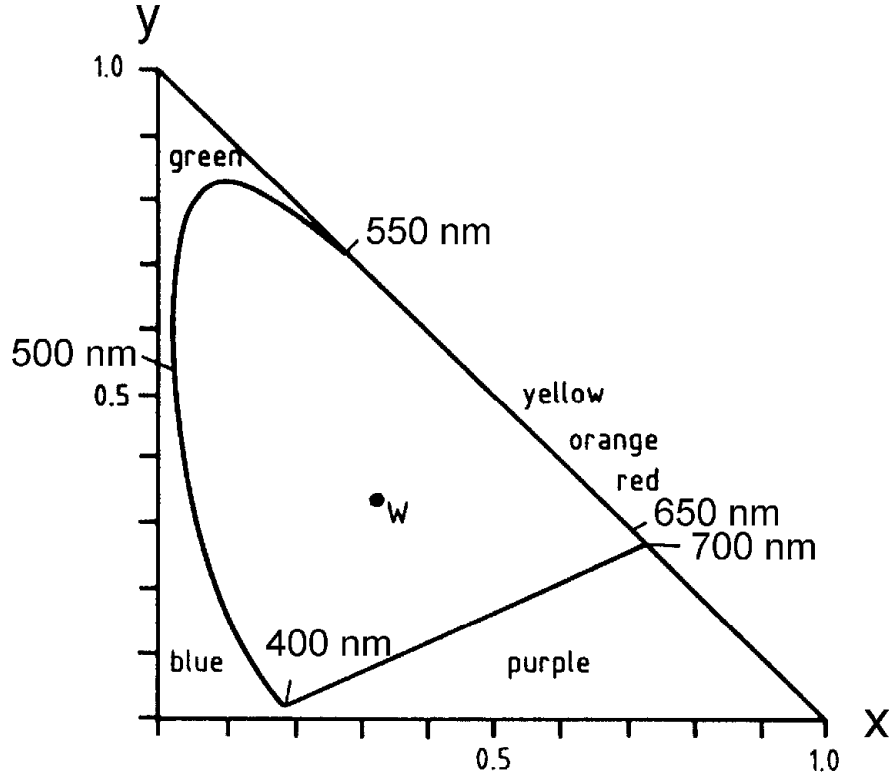


Figure 2. The CIE chromaticity diagram.⁸⁵ W = neutral point (white). The figure has been modified by the author. (© 1991 VCH).

In 1976 CIE introduced the improved CIELAB system and recommended it as a universal standard.^{83,85,88} In the CIELAB system, L, A and B refer to the three axes of space: L* representing the lightness axis, and a* and b* representing both the hue and the chroma of the red-green and blue-yellow axes, respectively (Fig. 3). L* may obtain values from zero for perfect black to 100 for white, which is the perfect reflecting diffuser. a* > 0 describes the redness, a* < 0 the greenness, b* > 0 the yellowness and b* < 0 the blueness of the colour.⁸⁹ The colour differences, ΔE, between two samples can be defined in terms of a difference in the lightness component ΔL, a difference in the chroma (saturation) component ΔC and a difference in the hue component ΔH:^{85,90}

$$\Delta E = [(\Delta L)^2 + (\Delta C)^2 + (\Delta H)^2]^{1/2} \quad (1)$$

In addition, ΔE may be defined as a total colour difference between a batch, B, and a standard, S:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (2)$$

where $\Delta L^* = L^*_B - L^*_S$, $\Delta a^* = a^*_B - a^*_S$ and $\Delta b^* = b^*_B - b^*_S$.⁹⁰ The hue difference (ΔH) can be obtained from the equation:⁹⁰

$$\Delta H = s[2(C_B C_S - a^*_B a^*_S - b^*_B b^*_S)]^{1/2} \quad (3)$$

where $s = 1$ if $a^*_B b^*_S < a^*_S b^*_B$ and $s = -1$ if $a^*_B b^*_S > a^*_S b^*_B$.

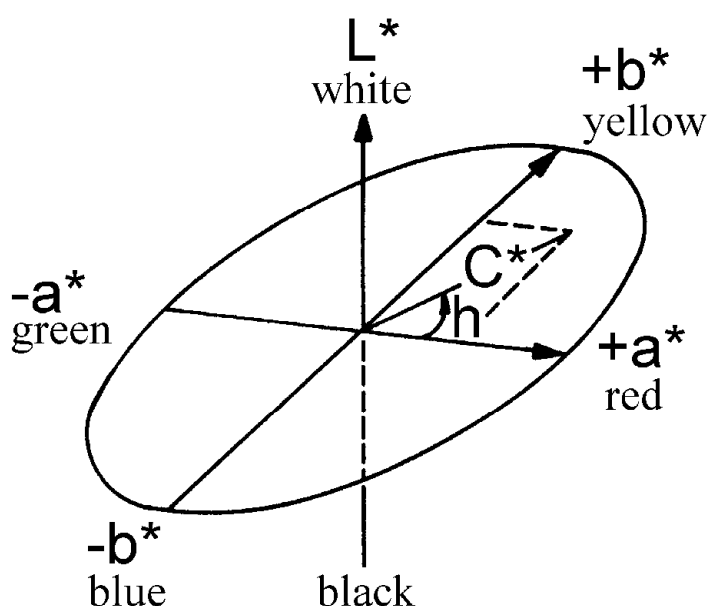


Figure 3. Rectangular and cylindrical co-ordinates of the CIELAB colour space.⁸⁵ L^* = lightness, C^* = chroma, h = hue angle, a^* = red-green axis, b^* = yellow-blue axis. (© 1991 VCH).

The first empirical approach to defining the constitution of a dye molecule was formulated by Witt in 1876. Witt's theory has remained as the basis of the subsequent empirical theories. Earlier, in 1868, Graebe and Liebermann had recognized that all dyes contain a system of conjugated double bonds.⁹¹ Witt's theory postulated that "a compound is coloured due to the presence of particular groups, chromophores, which must be linked to the system of conjugated double bonds. Potential chromophoric particles may become coloured if auxochromic groups are introduced".⁹¹ The linear or cyclic systems of conjugated double bonds in organic dye molecules are called

chromophores, whereas electron donors, *e.g.* NH_2 or OH groups, are called *auxochromes* and electron acceptors, *e.g.* NO_2 or Br groups, are called *antiauxochromes*. The structural system of these three components is called a *chromogen*, a colour-producing element.^{91,92}

The majority of organic dyes contain an extended, conjugated chromophore system to which electron donor and electron acceptor groups are attached.⁹² Absorption from the ultraviolet can be shifted into the visible region by increasing the size of the conjugated system. This can be achieved by extending the size of the chromophore or by adding donor or acceptor groups in positions where they directly extend the conjugation or where the p electron orbitals of the jointed groups are able to interact with the overall π system.⁹² Optimum overlap of the π -orbitals is essential and is achieved in coplanar molecules.⁹³ A shift of the absorption maximum to a longer wavelength is called a *bathochromic* shift, whereas shift to a shorter wavelength is called a *hypsochromic* shift. Increase and decrease of absorption are called *hyperchromic* and *hypochromic* changes, respectively.^{91,92}

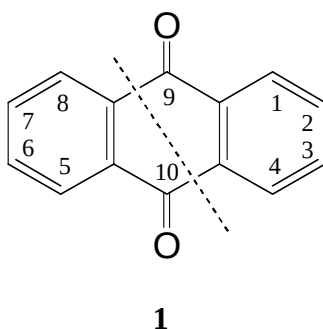
The energy of visible or UV light is absorbed by the molecule and used to promote electrons to higher energy levels, *i.e.* excited states.⁹³ The colour of an organic molecule is based on its properties of absorbing electromagnetic radiation at wavelengths 400–700 nm. Simple organic molecules have electronic transitions, which absorb in the UV region at wavelengths of less than 400 nm of the spectrum, and therefore have no colour.⁹² Valence Bond (VB) and Molecular Orbital (MO) theories, which are based on quantum mechanics, make quantitative descriptions of light absorption by organic compounds possible.^{93,94} VB theory is rarely used nowadays, because it is inconvenient and virtually impossible to apply to large molecules.^{94,95} The Pariser-Parr-Pople (PPP) model of the MO theory has been widely and successfully used for predicting the colours and tinctorial strengths of dyestuffs.^{93–95} Quantitative calculations are important in the synthetic design of colorants, because not only the wavelength of the absorption maximum but also the molar extinction coefficient, ϵ , play important roles.⁹³ Instead of using one dye to obtain a certain colour, a mixture of two or more dyes with different absorption spectra can be used. However, in that case the shade is always less brilliant than if only one dye is used for the colour.⁷⁴

2.3 Anthraquinone dyes

Anthraquinone dyes are the second important class of dyes, while the most important class consists of azo dyes, which form a little over 50% of the commercial colorants.^{96,97} The advantage of anthraquinone dyes compared to azo dyes is their superior brightness. Anthraquinone dyes possess good light-fastness properties, which makes metallization unnecessary. This is an advantage, because metallization dulls colours.⁹⁸

Anthraquinone dyes are used as reactive and vat dyes for cellulose fibres, as disperse dyes for polyester and as acid dyes for polyamide. In general, anthraquinone dyes and their intermediates have not been reported as strongly toxic substances. However, in tests with rabbits, most compounds caused mild irritation of the eyes.⁹⁹

The nature and the number of the substituents, as well as their position in the anthraquinone ring system, play important roles in the electronic absorption spectrum of the compound.¹⁰⁰ This property can be utilized when preparing synthetic anthraquinone dyes. Anthraquinone may be considered as consisting of two isolated benzoyl chromogens (**1**), in which the substituents, located in different rings, interact very little. Thus, most anthraquinone dyes are 1,4- or 1,2-disubstituted and almost any shade of a colour can be produced by incorporating two donor groups of different strengths in the 1- and 4-positions of the anthraquinone ring.¹⁰⁰ Compounds in which the two substituents are in different rings have only limited applications.^{100,101} In addition, the 1,4-substituted anthraquinones have much more bathochromic and hyperchromic spectra than have their 1,5- and 1,8-derivatives.^{100,101}



Natural pigments, for example anthraquinones, are produced by the secondary metabolism of organisms. Secondary compounds have no recognized role in the maintenance of fundamental life processes in the organisms that synthesize them, *e.g.* they do not serve as a source of energy or as a specific storage product.^{102,103} Secondary

compounds are not restricted to the plant kingdom, even though the great majority of these products have been isolated from plants and micro-organisms.¹⁰⁴ The main biological function of plant secondary metabolites is chemical defence. Furthermore, secondary products are of great importance to the reproductive life of plants and animals. They protect plants against attack by micro-organisms and animals, and increase the ability of plant species to compete with other species in a habitat. In addition to their ecological role, some secondary compounds may merely be the end products of aberrant biosynthetic pathways or they may be excretion products.¹⁰⁴ Three different possibilities for the storage of secondary products exists: products may be stored in the same cell where they are formed, they may be accumulated in cells adjacent to the cells of synthesis, or they are stored in organs, which differ totally from the location of their synthesis, making long-distance transport necessary.¹⁰³ A majority of the secondary compounds are synthesized in the cytoplasm or cytoplasmic organelles in cells. Most hydrophilic metabolites are stored in the central vacuole, which is well separated from the cytoplasm by means of tonoplast.^{102,103} Thus, the synthesized compounds have to be transported or diffused across the tonoplast against a concentration gradient, *i.e.* from their low concentration in cytoplasm into their high concentration in vacuoles.¹⁰³ Compounds can also be accumulated in the cell walls, and, if lipophilic in character, in special excretory cells or spaces.¹⁰² Secondary products are not inert end products but rather they are further metabolized and even degraded. Secondary product metabolism is a complex dynamic system, where synthesis and degradation are two partially linked processes, which are integrated into the complete network of plant metabolism.¹⁰⁵ It is believed that degradative enzymes, such as glucosidases, are not in the vacuole or associated with the inner tonoplast membrane. Hence, for degradation, the secondary products must be released from the vacuole to be turned over or further metabolized.¹⁰⁵ More about β -glucosidase enzymes can be read in the introduction of Publication I.

Natural anthraquinone compounds occur as aglycones as well as C- or O-glycosides with a great variety of colours from pale yellow to almost black. Compounds occur in many organisms, chiefly in higher plants, fungi, lichens and bacteria and, in the animal kingdom, in some insects.¹⁰⁶ Fungal anthraquinone pigments are found abundantly in *Dermocybe*.^{107,108} Natural anthraquinone dyestuffs, such as kermes and madder, have been used for decades to dye textiles.¹⁰⁶ In recent years, natural anthraquinones have been used as colouring agents for beverages, sweets and other

foods.^{109,110} Also, natural colorants, especially hydroxyanthraquinones, have been used to colour human hair.^{111,112} Natural hydroxyanthraquinones were suggested to be applied as isolated compounds, C- or O-glycosides or as plant extracts. It was discovered that hydroxyanthraquinones coloured hair directly even at room temperature and that these dyes were resistant to perspiration, washing, light and adverse weather conditions. Furthermore, hydroxyanthraquinones were very stable in solutions of the cosmetic media. Among many natural hydroxyanthraquinones, those occurring in *Dermocybe sanguinea*^{*}, like emodin, dermocybin and dermorubin, were mentioned as potential hair dyes.¹¹² More about the application of anthraquinones is explained in the introduction of Publication I.

Most natural anthraquinones are polyhydroxy or methoxy derivatives with little variation in skeletal structure.¹¹³ For the formation of anthraquinones, at least two biosynthetic routes have been reported. The most important is the acetate-malonate pathway, which includes suitable folding and condensation of a polyketide chain derived from acetate units (Fig. 4). The other biosynthetic route is the shikimate-mevalonate pathway, where the precursor is shikimic acid.¹¹³ Fig. 4 illustrates the biosynthetic relationships between the pigments of *D. sanguinea*, which are formed via the acetate-malonate pathway.¹¹⁴

Emodin (Fig. 4) is probably the most widely distributed anthraquinone and several synthetic methods have been reported for it.^{115–117} Most of the syntheses are based on biogenetic schemes. In the method of Harris *et al.*, β -polycarbonyl compounds were used as starting material. The reactions resulted in a 70% yield of emodin.¹¹⁶ In the fungus *D. sanguinea*, emodin- and dermocybin-1- β -D-glycopyranosides are the most abundant anthraquinones. For dermocybin, no syntheses have been proposed and, thus, its isolation from Nature is still the only way of obtaining the pigment. Syntheses have been developed for endocrocin and some other minor compounds found in *D. sanguinea*.^{114,118–120}

^{*} There is disagreement about the rank of *Dermocybe* in the systematical hierarchy, where it has been regarded as a subgenus of *Cortinarius* or a genus of its own. In most chemical publications *Dermocybe* has been regarded as a genus of its own and the same ranking is used here, although Scandinavian mycologists consider *Dermocybe* as a subgenus of *Cortinarius*. *Cortinarius sanguineus* = *Dermocybe sanguinea*.

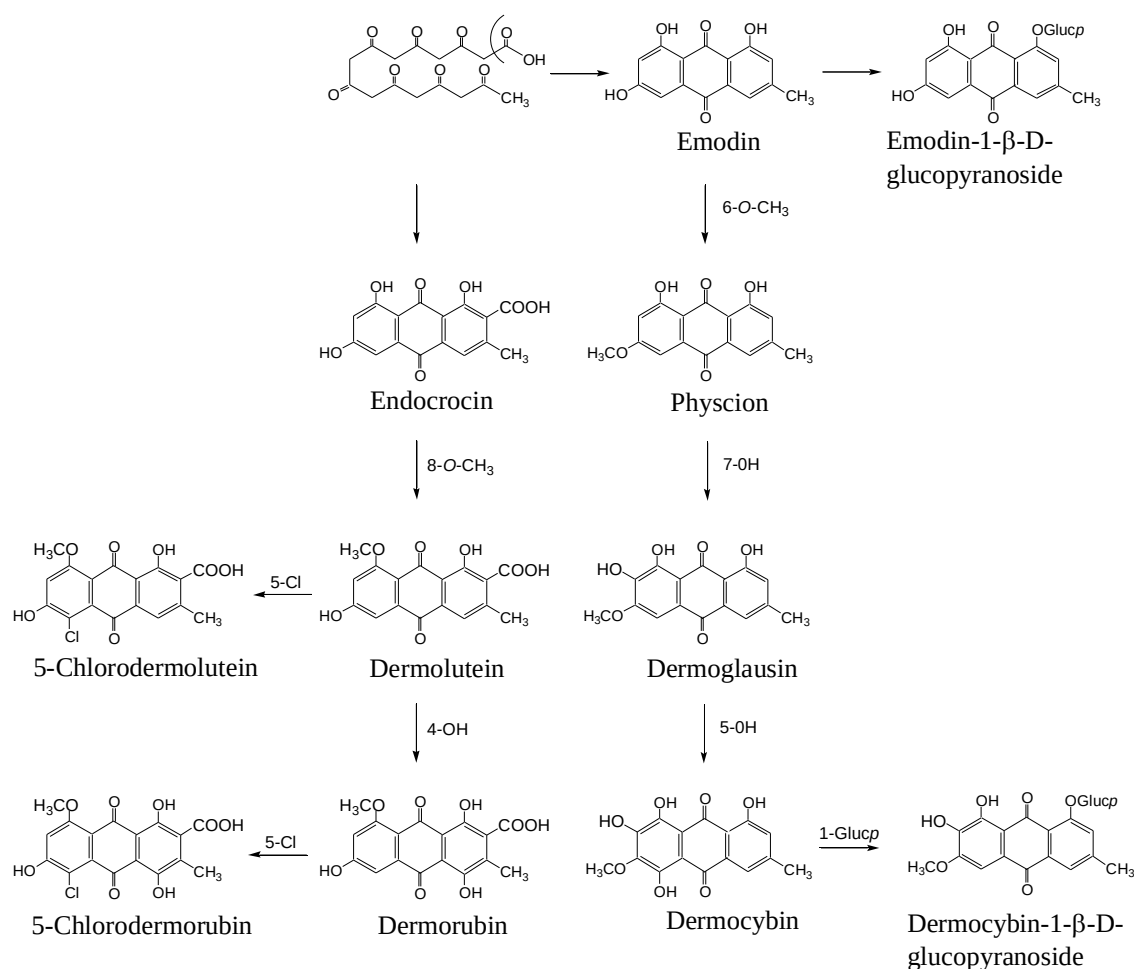


Figure 4. Biosynthetic relationships between pigments of *Dermocybe sanguinea*.¹¹⁴ (Reproduced with permission of the copyright owner, © 1987 Springer-Verlag).

2.3.1 Separation and structural analysis of anthraquinones

Natural anthraquinones and natural anthraquinone glycosides have a very strong tendency to form self- and mixed aggregates, on account of the presence of carbonyl, hydroxyl and carboxyl groups capable of forming intermolecular hydrogen bonds. Therefore, their complete separation is difficult and mostly requires the application of several different separation techniques. In a large-scale isolation, the problems with aggregation are even greater than in separation on an analytical scale. Apparently, the most common techniques for the separation of anthraquinones are high-performance liquid chromatography (HPLC), column chromatography (CC) and thin-layer chromatography (TLC).^{120–129} Preparative HPLC, CC and TLC methods are suitable for only relatively small amounts of compounds. Thus, to obtain large amounts of pure

compounds, repetition of the processes is necessary. Furthermore, it seems that the chromatographic methods have limited potentiality in separating samples containing several anthraquinone derivatives in very different ratios, because some of the derivatives overlap each other partially or completely. In addition, the adsorption of the compounds on the solid support seems to be a problem in chromatography.^{130,III}

To avoid the disadvantages of the HPLC, CC and TLC techniques, liquid-liquid partition methods have been used in separations.^{130–139,III} The liquid-liquid partition methods are based on two immiscible liquid phases. The compounds to be separated must have different partition coefficients between the two phases. Hynninen was among the first researchers to apply stepwise pH-gradient elution in liquid-liquid separations.^{133–135} Pure liquid-liquid partition methods offer the best techniques for the preparative separation of substituted anthraquinones.

Electronic absorption spectroscopy is an important method for the characterization of anthraquinone compounds. The unsubstituted anthraquinone has a pale yellow colour owing to a weak band in the visible region at *ca.* 405 nm.¹⁰¹ The increasing electron-donating ability of the substituents, *e.g.* OCH₃, OH, NH₂, NHCH₃ and N(CH₃)₂, leads to a bathochromic shift of the electronic absorption band.¹⁰¹ In the UV region, substituted anthraquinones show intense benzenoid absorption bands fairly regularly within the ranges 240–260 and 320–330 nm. The quinonoid bands appear in a range from 260 to 290 nm and hydroxyanthraquinones show an absorption band between 220 and 240 nm.¹⁴⁰ Most natural anthraquinones have complex structures with several substituents, which modify their absorption spectra. Thus, for example, multibanded and wide absorptions may be observed.¹⁴¹ Ionisation of a hydroxylic group results in a bathochromic shift.¹⁴² Electronic absorption spectra of some natural anthraquinones are shown in Publications II and V.

Loss of two molecules of carbon monoxide is a feature common to the mass spectra of anthraquinones. Substituted anthraquinones have additional peaks appropriate to their substituents; for example, methoxy compounds eliminate a formyl radical, –CHO. Losses of –OH and H₂O from hydroxyl derivatives may be observed and the cleavage of CO₂ is characteristic of a carboxylic acid compound.^{129,143} Fig. 5 shows the mass spectrum of the natural hydroxyanthraquinone dermocybin.

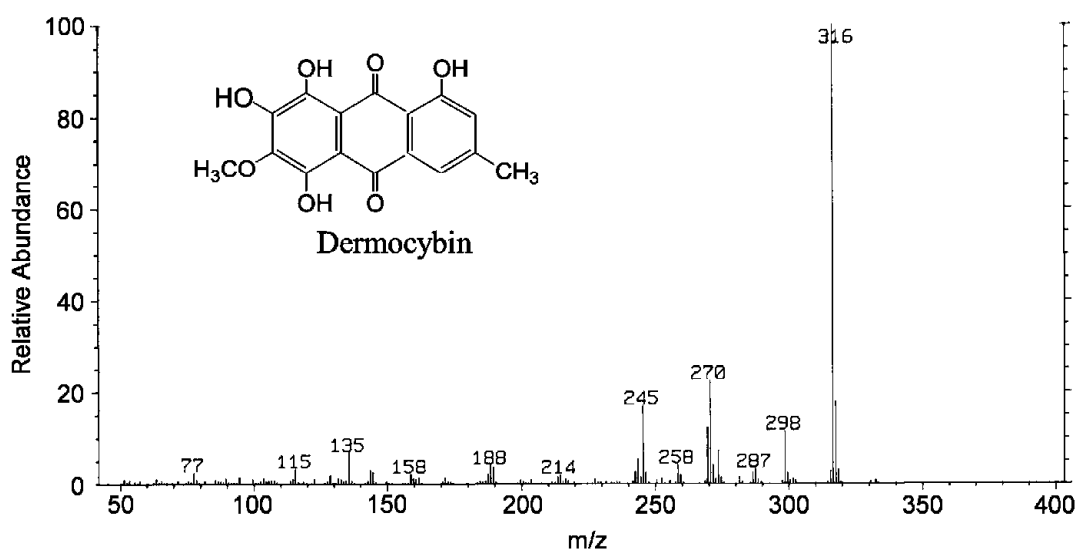


Figure 5. The mass spectrum of dermocybin, which shows the characteristic fragmentations of the hydroxyanthraquinone derivative. The mass conditions of positive-ion electron impact (EI) are explained in Publication II. 316 $[M]^+$, 298 $[M - H_2O]^+$, 287 $[M - CHO]^+$, 270 $[M - H_2O - CO]^+$, 258 $[M - 2 CHO]^+$.

2.3.2 The fungus *Dermocybe sanguinea*

D. sanguinea grows in conifer forests, especially in moist places with a well-developed thick moss layer (Colour Figs. 2 and 3 in Appendix 2). It is relatively common throughout Finland and Scandinavia, apart from the area above the Arctic Circle.¹⁴⁴ *D. sanguinea* is easily recognized by its throughout blood-red coloured cap, stem and flesh. The cap has a diameter of 2–5 cm, while the stem is 3–9 cm long and 3–8 mm thick.^{144,145} *D. semisanguinea*, a close relative of *D. sanguinea*, is observed to form reddish-brown mycorrhizae with pine, spruce, birch and willows.¹⁴⁶ Species of *Cortinarius* and *Dermocybe* seem to be rather difficult to cultivate, as is the case with most other mycorrhizal fungi.¹⁴⁵ However, there have been attempts to obtain aseptic cultures of *D. semisanguinea*. On Hagem agar, a reddish-brown mycelium, of a colour which matched that found in the fungus in nature, was observed to grow slowly.¹⁴⁶

Dermocybe species have been considered as non-toxic fungi.¹⁴⁷ In recent studies, however, they have been found to contain traces of renal toxins, such as those existing, for example, in *Cortinarius speciosissimus* in subgenus *Leproclybe*.^{148,149} Because, *Dermocybe* species contain large quantities of anthraquinone pigments as glycosides or aglycones, they are regarded as potentially toxic fungi. Some anthraquinones, such as

emodin and its chloroderivatives, have been found to have slightly toxic or carcinogenic properties.^{145,147,150–152} Unfortunately, only a few natural anthraquinones have been tested. Thus, which untested anthraquinones are toxic and which not and whether the results of one study, such as Ames' test, give reliable information about the toxicity to man cannot be stated with certainty.^{149,152} Apparently, the anthraquinone pigments in *Dermocybe* protect the fruit bodies from insects, as it has been observed that larvae are seldom found within the fruit bodies.¹⁴⁷

2.4 Natural dyeing in Finland

In Finland, cremation prevailed as a burial custom up to the 11th century and, therefore, many of the oldest textile fragments were totally destroyed. From the inhumation burials, *i.e.* graves, of the later Iron Age[†] a great many textiles with marks of dyes have remained. The use of dyed textiles was common in Finland by the end of the Viking Age at latest.^{153,154} The predominant colour was blue, followed by red and green.^{153,154} Blue dye was obtained from woad (*Isatis tinctoria*), which was known in the eastern Baltic countries from the Late Iron Age and which has been found along the coast of the Gulf of Finland and the Turku archipelago.^{154,155} As woad was a weed among grain in its natural distribution area in Eastern Europe, it seems likely that the spread of woad in the coastal areas was connected with the grain trade, shipwrecks *etc.*¹⁵⁵ Blue dye might also have been obtained from the indigo plant. Because indigo possesses all the necessary properties of an object of long-distance trade, for example high price, it seems fairly plausible that, during the Viking Age, indigo of southern origin was available in the Baltic countries.¹⁵⁵ The most important sources of red dye in prehistoric Finland were the roots of various *Galium* species. In addition, yellow dye was obtained from the stem and leaves. Yellow dyestuff was also extracted from *Lycopodium* species or lichens.¹⁵⁴

Since the majority of the archaeological textiles have been found in graves, they mainly represent festive textiles. Apparently, blue colour had a significant position in the ancient fashion – it was a luxury that only the prestigious of society could afford.

[†] Iron Age ~ 500 BC–1000 AD, Middle Ages ~ 500 AD–1500 AD, Viking Age ~ 800 AD–1100 AD.

Woad thrived only in the islands of the Baltic Sea, and since it could not be cultivated inland, goods had to be acquired through merchants.¹⁵⁵ According to the predominance of the blue textile findings, it is also good to bear in mind that the colour fastness of indigo (the main colouring component in woad) is superior compared with red and especially yellow dyes (Table I).^{45,156}

Alum was the mordant most widely used in Europe during prehistoric times and the Middle Ages. It was brought to Europe mainly from Asia Minor. Some plants, *e.g.* lichens and *Lycopodiaceae*, contain biologically accumulated aluminium and it is possible that such plants were used for mordanting. However, the assortment of dye plants and dyestuffs used for textiles in those times was relatively small. It is likely that the difficulties in obtaining mordants and their presumably high prices were the reasons why new dyeing experiments were not performed.¹⁵³

Later, imported dyestuffs such as cochineal, madder and indigo became more and more important. Until the latter part of the 19th century, imported dyestuffs were predominant in professional dyeing.¹⁵⁷ Because the exported dyestuffs were expensive, attempts were made to find indigenous dye plants and to grow foreign species. Even the state authorities encouraged carrying out such experiments. Professor Pehr Adrian Gadd of the University of Turku made trials of growing foreign dye plants. He cultivated safflower, madder, weld, woad and goldenrod (*Solidago canadensis*).^{158,159} In Sweden, Westring surveyed the dyeing properties of different lichens.³³ Apparently, the only note of the use of fungi for dyeing experiments by the Swede Linder may originate from the same sort of dyeing experiments. In his book, Linder mentions old *Lactarius* (milk cap) as a source of yellow colour.³² Possibly, the colour obtained was not satisfactory and no further experiments were made with fungi.

In the late 19th century, synthetic dyes became prevalent. However, their colours were very different from the natural ones previously used and the bright shades aroused criticism and debate in the cottage industry.¹⁶⁰ In addition, the colour fastness properties of the first synthetic dyes were mostly poor and, thus, natural colorants were preferred in crafts. Suomen Käsityön Ystävät (SKY), which was founded in 1879 to maintain national textile traditions, proceeded to spread knowledge of natural dyeing.^{160,161} SKY organised dyeing courses all over in Finland and the first book on natural dyeing, written by Aline Hellén, head of the dyeing department in SKY, was published in 1905.^{42,160}

In recent years, natural colorants have gained increasing interest commercially, as the use of renewable natural materials has become more common. According to the publications, natural dyeing projects are going on in several parts of the world aiming at the maintenance of the living craft culture and, on the other hand, aiming at the wider use of natural dyes. Interdisciplinary projects have been launched to develop production and refining of natural colours. A project, financed by the European Union (EU), called “SPINDIGO – Sustainable production of plant-derived indigo” started in spring 2001 in several European countries, *i.e.* England, Germany, Spain, Italy and Finland. The aim of the project is to study and improve the cultivation techniques of the indigo-producing plants *Isatis tinctoria*, *I. indigotica* and *Polygonum tinctorum* and also to develop a simple method for the isolation of indigo. Dyeing techniques are also to be studied, the purpose being to standardize them. The main object of the SPINDIGO project is to develop the cultivation of new non-food plants in Europe and thus, to provide new sources of occupation in the countryside.¹⁶² Another EU project, coordinated by the EVTEK Institute of Art and Design at the Espoo-Vantaa Institute of Technology, is to develop printing techniques for natural colorants.¹⁶³ These projects are developing methods to enable a wider use of natural dyes, even aiming at industrial scale. A few master’s theses and minor educational studies concerning natural pigments have been made. In these studies the traditional or home-dyeing recipes were applied, and water extracts of different plants were used as dye liquors for natural fibres.^{1,164–169} In one study, natural dyeing recipes were drawn up as educational material for a school in Tanzania, Africa.¹⁷⁰

The study of anthraquinone pigments occurring in *Dermocybe sanguinea* began in 1980’s at the University of Kuopio. Professor Paavo H. Hynninen, being interested in organic natural products, started the research inspired by two craft workers, Kaija Jauhiainen and Lahja Hakala. In the thesis for master’s degree, Eila Nokelainen (the student of Prof. Hynninen) studied the anthraquinone glycosides and anthraquinone aglycones of *D. sanguinea*. For the analytical separation of these pigments, she developed the 1D TLC using silica plates and toluene-ethyl acetate-ethanol-formic acid (10:8:1:2, v/v/v/v) as eluent. In addition, Nokelainen made some ionisation measurements and determined pK_a values for emodin, emodin-1-β-D-glucopyranoside, dermocybin and dermocybin-1-β-D-glucopyranoside in methanol.¹⁷¹ The results of Nokelainen have been in a great value for this study. Also, some preliminary experiments on dyeing were performed. Wool and cotton were dyed using extracts from

dried or frozen fruit bodies of *D. sanguinea* as dye liquors. These extracts were mixtures of anthraquinone aglycones and anthraquinone glycosides.^{172,173} This research has been performed inspired by the promising results of the preliminary investigations. It introduces fungi as a source of natural dyestuffs. Fungi are more rarely used in natural dyeing, even though some species are known to possess stable colorants. The large-scale utilization of natural colorants and, for example, textile printing require that colorants can be obtained in powder form. Since fungi are structurally and functionally, *e.g.* they do not have photosynthesis, simpler organisms than higher plants, the isolation of secondary metabolites, *i.e.* pigments, from them seems to be quite easy. The isolation procedures described in Publication I can be useful both for craft workers and for industry.

Apparently, natural colorants lie in the centre between the past and the future. They were the first dyes to be utilized for colouring materials. Hence, they are the part of history, and, in some forms, have survived till these days as a live craft culture. Natural dyes also have opportunities for future development. Pigments could be isolated from plants, algae¹⁷⁴ and fungi, using advanced techniques, and these pigments could be processed further by chemical means. Cultivation, genetic engineering, enzyme technology and cell cultures offer a wide range of possibilities for future developments.

3 QUALITY OF DYED PRODUCTS

The properties of the products have to fulfil the requirements of the consumers. Even in past centuries, the textile trade required quality control, testing and regulations.¹⁷⁵ In order to facilitate international coordination and the unification of industrial standards, the organization of ISO (International Organization for Standardization) was founded in 1947. ISO is a worldwide non-governmental organization in which some 140 countries participate.¹⁷⁶ Even very early on, it was realized that standardization would help to diminish technical barriers to trade and so to rationalize the international trading process. Furthermore, international standards tend to make life simpler by increasing the reliability and effectiveness of goods and services. The first ISO standard was published in 1951 and ever since they have been important for trade as well as for consumers.¹⁷⁶

One of the important properties of dyed material is the fastness of the colour. The level of colour fastness desired in a product is determined primarily by its intended use. Low light-fastness may be acceptable for dyes used to colour paper, provided that the colorants are inexpensive and the lifetime of such products is short. In contrast, excellent light fastness is demanded for furnishing fabrics, since they are usually expected to last for many years. In most cases, textile dyes fall in between these two extremes.^{177,178} Consumers are aware of factors, such as cost, expected wear life and preferred methods of cleaning, which affect their satisfaction with a product, but these factors are often connected with the level of colour fastness exhibited by the item.¹⁷⁹ Colour fastness^{180–182} is usually evaluated by comparing the colour differences in a fabric before and after a test with the lightness differences of the grey scale (or blue scale in the case of light fastness).^{183,184} The evaluation is visual. However, it has been noticed that the values for colour fastness differ between different assessors and that, even between repeated assessments by one and the same assessor, they may vary by as much as three points.^{185,186} Thus, instrumental assessment methods for colour fastness have been developed.^{88,185–189}

Light fastness is among the most important properties that must be satisfactory for textile dyes. Generally, natural anthraquinone dyes have good light-fastness properties.¹⁵⁶ Colour fastness of natural yellow dyes is mostly poor.^{45,156,190} On fading, most samples show loss and dulling of the colour, accompanied by a change of hue.¹⁹⁰ Most natural dyes initially fade rapidly, then continue to fade at a slower rate. Most of

the light-fast natural dyes, such as the anthraquinone dyes cochineal and madder, fade gradually at a constant rate.¹⁵⁶

On wool, natural dyestuffs generally have moderate fastness to washing, as evaluated by the 2A ISO test. Logwood and indigo are exceptional in this respect, as they receive high values for washing fastness.¹⁹¹ A significant number of natural dyes, when treated in weakly alkaline solution, show marked changes in hue. Usually, natural compounds contain OH groups, the deprotonation of which is pH-dependent. In many cases, ionisation has an effect on the colour of the compound, as it causes a bathochromic shift in the electron absorption spectrum of the dye molecule.^{142,V} The tendency to change colour makes it necessary to have accurate knowledge of the pH of alkaline solutions for the cleaning of textiles dyed with natural dyestuffs.¹⁹¹ Apparently, repeated washings under mildly alkaline conditions do not have a cumulative effect and therefore, after several washings, textiles reach a state of stability.¹⁹¹

Fastness tests for light and for washing are the most important and most frequently used methods for testing textiles in normal use. In addition, there are several other properties of colour fastness which may be determined, for example rubbing fastness, fastness to sweating, seawater, chlorination and bleaching.¹⁷⁸ In rubbing fastness, the surface properties of the dyed materials play an important role.

4 FIBRES

This section explains the structures of the textile fibres used in the experimental part of the study. Natural and synthetic polyamide fibres, *i.e.* wool, silk and polyamide, are considered. The structures of cellulose fibres differ considerably from those of polyamide fibres and thus, they are not included. In addition, the results of the preliminary dyeing experiments were poor for cotton,^{172,173} which confirmed the decision to exclude the cellulose fibres. Polyester was chosen for disperse dyeing experiments, because it is one of the most commonly used disperse-dyed textile fibres.

4.1 Wool

Wool is commercially the most important animal fibre. Not only do different wool fibres vary greatly in diameter and length, but they also vary in their physical properties, colour, crimps and scales. Wool belongs to the family of proteins called keratins. Keratin fibres are chemically heterogeneous and can vary significantly in amino acid composition, not only among fibres from different individuals of a single species but even along the length of a single fibre from the same animal. The differences in amino acid composition are influenced by genetic origin, physiological state and nutrition.¹⁹² Eighteen different amino acids have been found to exist in wool (Table II).^{192,193} These amino acids form 170 different types of polypeptides varying in molecular mass from under 10 000 to more than 50 000.¹⁹³

Because the polypeptide chains are ramified, they are unable to lie close to each other and no organised structure can be formed. However, adjacent polypeptide chains are linked to each other by covalent and non-covalent interactions making up inter-chain linking. Chemical bonds can also occur between different parts of the same polypeptide chain, which means that intra-chain bonds are formed. Inter-chain bonds are more important than intra-chain bonds where the properties and performance of wool are concerned.¹⁹² As a result of intra- and inter-chain linking, the polypeptide chains are coiled up like a spiral staircase, which is called α -keratin. When the fibre is stretched, the coil extends to β -keratin. When the tension is released, the cystine and other inter-chain crosslinks pull the keratin back into the α -form. This ability to stretch and retract is important and accounts for the resilience of wool.¹⁹⁴

The sulphur-containing amino acid cysteine plays an important role in wool. The cysteine residues in the fibre are linked in pairs, providing a covalent disulphide bridge, –S–S–. These covalent crosslinks can be formed either between different protein chains or between different parts of the same protein chain (Fig. 6). The disulphide inter-chain crosslinks are responsible for the greater stability and lower solubility of keratin compared with most proteins.^{192,193} Covalent isopeptide crosslinks are also present in wool (Fig. 6).¹⁹²

Table II. Amino acid residues of wool and silk in percentage units.^{192,193,202}

Amino acid	Wool %	Silk (<i>Bombyx mori</i>)	
		Fibroin, %	Sericin, %
Alanine	5.3–5.4	30.0	5.8
Arginine	6.8–6.9	0.5	3.1
Aspartic acid and Asparagine	6.4–6.6	1.9	14.6
Cysteine	10.0–10.5	trace	0.1
Glutamic acid and Glutamine	11.9	1.4	6.2
Glycine	8.2–8.6	42.9	13.5
Histidine	0.8–0.9	0.2	1.4
Isoleucine	3.1	0.6	0.7
Leucine	7.7	0.6	0.7
Lysine	2.8–3.1	0.4	3.5
Methionine	0.4–0.5	0.1	0.1
Phenylalanine	2.8–2.9	0.7	0.4
Proline	5.9–7.2	0.5	0.6
Serine	10.3–10.5	12.2	34.0
Threonine	6.3–6.5	0.9	8.8
Tryptophan	0.5		
Tyrosine	3.7–4.0	4.8	3.6
Valine	5.5–5.7	2.5	2.9

For non-covalent interactions, hydrogen bonds, ionic bonds and hydrophobic interactions are the main forms (Fig. 6). Ionic bonds are formed between basic amino

and acidic carboxyl groups. These groups are responsible for the amphoteric character of the fibre and its ability to combine with acids or bases. In the isoelectric state, the net charge on the NH_3^+ and COO^- groups in the fibre is zero. The isoelectric point is stated to be around the pH 4.5.¹⁹⁴ Thus, at pH values below 4.5, the net charge of the fibre is positive whereas at the pH values above 4.5 there are more negative charges.^{192,194} Because of its amphoteric nature, wool can be dyed under acidic or basic conditions. However, strongly basic conditions are unsuitable for dyeing, because, under such conditions, the chemical structure of the wool will break down. The level of damage to the wool fibre is kept to a minimum when wool is dyed at a pH value within the isoelectric region, which means at pH 4.5–8.^{194,195}

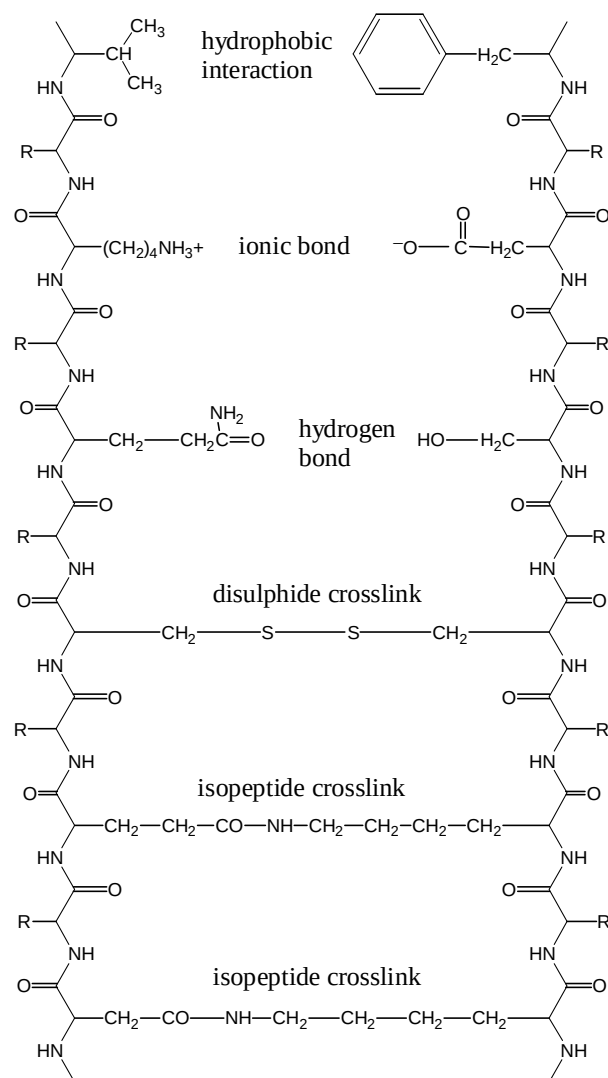


Figure 6. Chemical structure and bonds in wool fibre.¹⁹² (Reproduced with permission of the copyright owner, © 1992 Society of Dyers and Colourists).

The morphological structure of wool is shown in Fig. 7. Wool contains cells of two types. The external cells are called the cuticle and the internal cells the cortex. The flattened overlapping cuticle cells, or scales, form a protective sheath around the cortical cells. In coarse wool fibres, there is a third type of cells called the medulla, which is a central core of cells arranged continuously or intermittently along the fibre axis. Air-filled spaces lie between the medullary cells.^{192,196} Cuticle cells are separated from the underlying cortex by the cell membrane complex, which also separates the individual cortical cells from each other. The cell membrane complex forms the only continuous phase in wool.^{192,194}

The cuticle cells constitute the outermost surface of the wool fibre. They determine many properties, such as wettability, tactile properties and felting behaviour.^{192,196} The cuticle may be further divided into four layers: the epicuticle, the exocuticle-A, the exocuticle-B and the endocuticle.¹⁹²

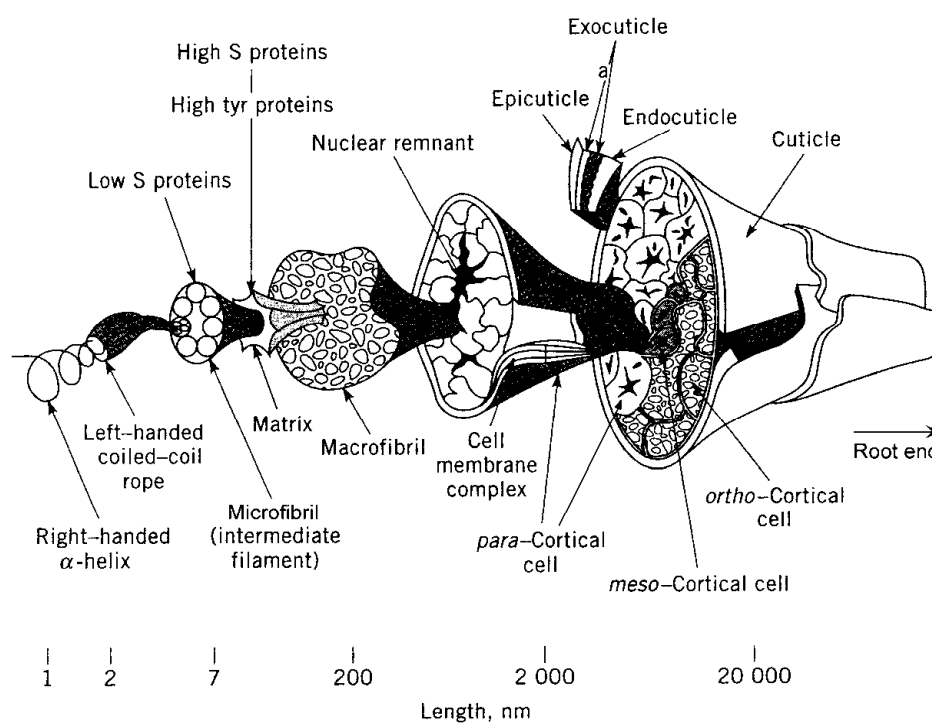


Figure 7. Schematic structure of a wool fibre.¹⁹⁶ (Reprinted with permission of the copyright owner, © 1998 John Wiley & Sons).

About 90% of the cortex consists of keratin fibres and is mainly responsible for the mechanical properties. Cortical cells are long, polyhedral and spindle-shaped, surrounded by a cell membrane. Cortical cells consist of intermediate filaments, also

termed microfibrils, which are grouped together into large macrofibrillar units. Cortical cells are divided into ortho- and paracortical cells according to the distribution of nonkeratinous material within the cell. Paracortical cells are clearly outlined and nonkeratinous material lies in specific regions called nuclear remnants (Fig. 7). Orthocortical and paracortical cells differ in the composition and arrangement of the microfibril-matrix system within each macrofibril. The relative proportions and arrangement of the ortho- and paracortex differ among wool types. In fine wool, for example, the two cortexes twist around the fibre and the orthocortex is always oriented towards the outside radius of the crimp curl. Apparently, the orthocortex is more accessible to the reagents and more chemically reactive than the paracortex.^{192,196} Acid, mordant, metal-complex and reactive dyes are usually used for dyeing wool.^{197,198}

4.2 Silk

Silk fibre is obtained from the cocoon of the silkworm. Silkworms have been cultivated in China since 2600 BC and probably uncultivated cocoons were collected and used long before that.^{199,200} The commercially most valuable cultivated species is *Bombyx mori*. Silk is also obtained from the fibres of the free-range wild silkworms, for example from the cocoons of the *Antheraea* species.^{199,200} The *Antheraea* cocoons are irregular in size and darker in colour than are the cocoons of the cultivated *Bombyx*.²⁰⁰ Fabric made from the silk of the wild *Antheraea* species is called tussah. The thickness of the warp and weft yarn in tussah varies greatly, giving the surface of the fabric an uneven look.²⁰¹

Silks differ in properties, composition and morphology, depending on the source. The fibre consists of the solidified viscous fluid excreted from the glands of the silkworm. The silkworm spins the whole of its cocoon without stopping and therefore a continuous thread is formed. One cocoon may contain as much as 2700 m of silk fibre, and about one thousand cocoons are required to produce 100 g of useful thread.¹⁹⁹ Silk contains two structural fibroin filaments coated with a family of glue-like sericin proteins, which result in a single thread with a diameter of 10 to 25 μm .²⁰² When producing silk thread, the sericin is removed and only the fibroin protein is used. Fibroin, containing 17 amino acids, is a less complex protein than keratin. Nearly 90% of the weight of the polymer consists of the four amino acids: glycine, alanine, tyrosine and serine.²⁰⁰ Table II (in Part 4.1) summarises the amino acid composition of the silk

of *Bombyx mori*. Since silk and wool are both protein fibres, they are dyed similarly. The dyestuffs primarily used for silk are acid and reactive dyes.¹⁹⁸

4.3 Polyamide

Polyamides, also called nylons (discovered by W. H. Carothers), are polymers which contain the amide repeat linkage in the polymer backbone.²⁰³ The first attempts to synthesize polyamide were made in 1899, but it was not until 1938 that the first commercially feasible synthesis was developed.²⁰³ There are two types of polyamides, which can be referred to as AB and AABB (Fig. 8). The AB polyamide type has all the amide linkages oriented in the same direction along the backbone, whereas for the type AABB the amide linkages alter in orientation along the backbone. The AB polymer can be formed in a polycondensation reaction from ω -amino acids and the type AABB can be formed from diacids and diamines.²⁰³ Hydrocarbons in the polymer can be aliphatic, aromatic or mixed. Polyamides which contain predominantly aromatic hydrocarbons are called aramids and their chemical and physical properties differ drastically from those of the regular polyamides, *i.e.* nylons. Type AB is called nylon- x (or PA- x), where x refers to the number of carbon atoms between the amide nitrogens. For type AABB, called nylon- x,x (or PA- x,x), the number of carbon atoms in the diamines is indicated first, followed by the number of carbon atoms in the diacid units.²⁰³

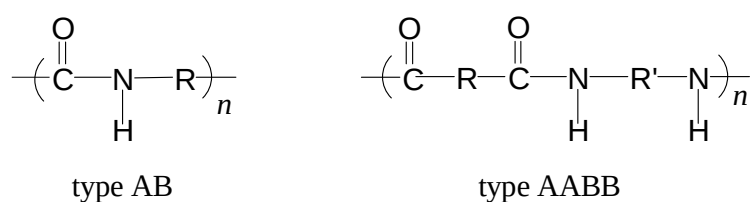


Figure 8. Structures of types AB and AABB polyamides.²⁰³ R and R' = aliphatic, aromatic or mixed hydrocarbons. (Reprinted with permission of the copyright owner, © 1996 John Wiley & Sons).

The most important polyamides are nylon-6 and nylon-6,6. Linear polymers consist of crystalline and amorphous phases. Crystallinity enhances, for example, hardness, abrasion resistance, tensile strength and elasticity, but decreases moisture absorption and strength on impact. Most commercial nylon-6 and nylon-6,6 fibres are 40–50% crystalline by weight.²⁰³ Because polyamides are melt-spun fibres, they may be

produced in a variety of cross-sectional shapes.²⁰⁴ Acid, mordant, disperse, direct and reactive dyes are most abundantly used to dye polyamides.¹⁹⁸

4.4 Polyester

W. H. Carothers discovered polyesters in 1929. He used linear aliphatic $\alpha\omega$ -glycols and dicarboxylic acid in his synthesis. The first polymers had low melting points and so were unsuitable for spinning. Ten years later Whinfield and Dickson discovered the polyethylene terephthalate, which was suitable for spinning. Commercial polyester fibres, such as Terylene, Dacron and Trevira, are condensation products of ethylene glycol and dimethyl terephthalate or terephthalic acid (Fig. 9).^{205,206}

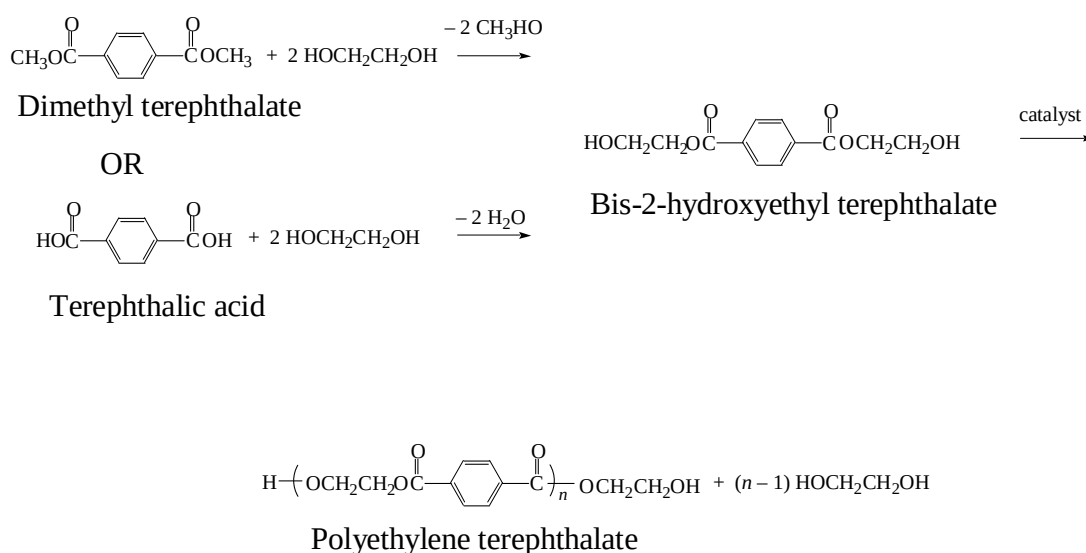


Figure 9. Synthesis of polyester.²⁰⁶ (Reprinted with permission of the copyright owner, © 1993 John Wiley & Sons).

Many product variants can be developed from the standard polyethylene terephthalate by controlling the molecular weight of the polymer, modifying its composition by using co-monomers and using additives such as delusterants, pigments or optical brighteners. Most textile fibres are delustered with TiO_2 to reduce the glitter and plastic appearance. By altering the cross section of the polymer by spinning with specially designed spinneret capillaries, the visual and tactile aesthetics of the fabric can be changed.²⁰⁶

The relative amounts of amorphous and crystalline structures in polyester fibres have a significant effect on their performance and properties. PES is highly hydrophobic and thus, difficult to dye. Normally disperse dyes are used, but there are modified fibres which can be dyed, for example, with basic or acid dyes.^{205,206}

5 DYEING MECHANISMS

The dyeing process can be considered as the distribution of a dye between two phases: the dye solution and the fibre. The process can be described by means of dyeing kinetics and dyeing equilibria. The distribution processes may be followed by chemical reactions in the fibres, which render the water-soluble dye ions or molecules immobile.²⁰⁷

The dyeing process consists of three principal stages, any of which may control or influence the rate at which dye molecules are transferred. These stages are the diffusion of dye through the dyebath to the surface of the fibre, the adsorption of dye molecules at the surface of the fibre and the diffusion of the dye from the surface of the fibre into its interior.^{11,192,207–209}

The development of the interface between the dyebath and the polymer molecules of a fibre is important for understanding the dyeing process. Most fibres have an enormous surface area relative to their mass, *e.g.* wool has a surface area of 100 m²/g. The external surface area of the fibre is only about one-thousandth part of the total surface area, which also consists of the walls of internal pores. When a fibre is placed in the dyebath, water penetrates the pores very rapidly, whereas, for the large dye molecules, penetration takes much longer. Within a short time, however, the dye solution is in contact with the internal surface. This means that an interface is formed between the polymer and the solution.²¹⁰

The fibre surfaces in contact with aqueous solutions are generally negatively charged, because anions in the solution are adsorbed on the surfaces. Cations are usually more hydrated than anions and thus they remain in the bulk solution. The anions, being less hydrated and more polarized, have a high tendency to be adsorbed. The ions will bind to the fibre surfaces through electrostatic attraction. Once a fibre immersed in aqueous solution receives a negative surface charge, it is surrounded by ions of the opposite sign, which concentrate in the solution near the surface, while ions of similar charge are repelled. As a result, close to the negatively charged surface there is an excess of counterions over co-ions. This difference in ion concentrations decreases with increasing distance from the surface, until in the bulk solution the concentrations of negatively and positively charged ions are equivalent and electrical neutrality is maintained. As a result of the concentration difference between negatively and positively charged ions, an electrical potential ψ exists in the surface layer. ψ decreases

to zero in the bulk solution. The above phenomenon is known as the electrical double layer; it is shown in Fig. 10.²⁰⁸

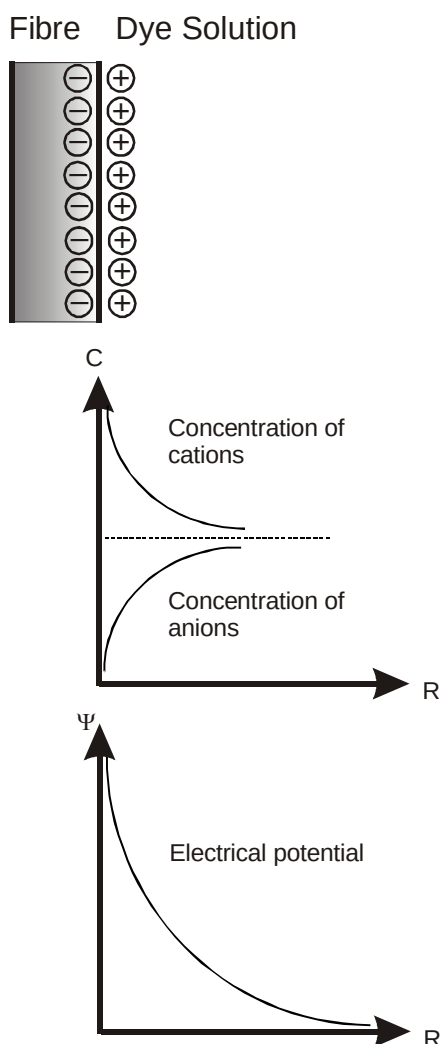


Figure 10. The electric double layer. C = concentration, R = distance from the fibre surface, Ψ = electrical potential.²⁰⁸ The figure has been modified by the author. (Reproduced with permission of the copyright owner of the book, © 1989 Society of Dyers and Colourists).

Electro-potential forces, temperature and agitation drive dye molecules towards the fibre surface.²¹¹ In dyeing, the free energy of the solution is greater than the free energy of the fibre phase. The difference in free energy is the driving force transferring the dye molecules from the solution to the fibre until equilibrium is reached and the free energies in the liquid and solid states are equal.^{207,211} Because equilibrium involves a decrease in the total free energy of the system, heat is liberated during dyeing and the reaction is exothermic. As a result of the exothermic reaction, the total amount of dye taken up by the fibre at equilibrium is less at higher than at lower temperatures.²¹¹

However, the time required to attain equilibrium is very long at low temperatures although rapid at high ones, because high temperature activates the molecular vibration and agitation in the solution. In practice, a compromise has to be made between the maximum degree of exhaustion of the fibre and the economy in processing time. Thus, to obtain adequate exhaustion within one hour, a temperature of at least 70°C is required.²¹¹ The mechanically caused turbulence of the dye liquor is important for agitation of the molecules.^{207,211}

Diffusion is the process by which matter is transferred from one part of a system to another as a result of random molecular motion.²⁰⁹ The following expression is known as Fick's law and it gives a quantitative value to diffusion. In the case of dyeing, it applies best to the diffusion of dye molecules from the outside layers into the interior of the fibre at the very beginning of the dyeing process.^{207,209,211}

$$ds/dt = -D(dc/dx) \quad (4)$$

where s = an amount of dye diffusing across a unit area,

t = time,

c = concentration of dye at a specified point x ,

x = the distance diffused and

D = the diffusion coefficient.

The rate of stirring of the solution can affect the rate of diffusion into a substrate by influencing the rate at which the molecules diffusing in the solution are brought to the surface of the solid phase.^{207,209} Diffusion, which takes place by random motion from regions of high concentration to regions of lower concentration, increases with a rise in temperature, as a consequence of a reduction in the time spent by a molecule in the vicinity of a reaction site or on the surface of capillaries.²⁰⁹ In the dyeing process, diffusion is always accompanied by adsorption of dye molecules.²⁰⁹

Adsorption of dye molecules at the surface may occur in two different forms: as physical adsorption through forces of physical attraction and through chemical adsorption, when bonding between neighbouring atoms is possible.²⁰⁸ When an undyed piece of material is placed in a suitable dyebath, uptake of dye is at first relatively rapid, but the rate decreases until eventually no further dye is adsorbed. However, the system is not in a static condition. On the contrary, the rate of adsorption of dye by the fibre is

equal to the rate of desorption from the fibre and thus, a state of equilibrium is achieved.²¹²

For a dilute solution of concentration c , Gibbs' adsorption isotherm may be written as^{208,210,211}

$$\Gamma = -\frac{c}{RT} \cdot \frac{d\gamma}{dc} \quad (5)$$

where

Γ = the excess concentration of solute in the surface layer
(or concentration of solute adsorbed at the surface),

$d\gamma/dc$ = the rate of change of the surface tension of the solution
with the increase in concentration of the solute

R = the gas constant

T = the absolute temperature

According to the adsorption equation, if the surface tension of the solvent is reduced by the solute, *i.e.* if $d\gamma/dc$ is negative, then the solute molecules will concentrate at the surface indicated from a positive Γ value. In contrast, if the surface tension is increased, which means that $d\gamma/dc$ is positive, then the Γ value will be negative, and the solute will move away from the surface into the bulk of the solution.^{208,210} Most water-soluble organic compounds, such as dyes, reduce the surface tension of water and therefore they concentrate at the surface. Highly hydrated substances, for example organic sugars (*cf.* dye glycosides in Table I), increase the surface tension and therefore move away from the surface to be more readily surrounded by water molecules.²¹⁰ In either case the solute tends to concentrate in the region of lower energy. According to above, it seems that dye glycosides would not be very suitable for dyeing, because as sugars they would rather stay in the solution.

There are several theoretical models for describing the adsorption equilibrium in dye-fibre systems. Langmuir and Freundlich equations were originally applicable to the sorption of gases by solids, and the Nernst equation was applicable to the distribution of solutes between two immiscible solvents.²⁰⁷ These theories have been modified further to give understandable descriptions to the dyeing processes. Donnan investigated the distribution equation for salt solutions.^{207,208} The Donnan theory of membrane equilibrium considers the distribution of ions on the two sides of a semi-permeable membrane.²¹² Donnan theory has been used successfully to predict the changes in dye

solutions, many of which consist of negatively and positively charged particles. The model is used to describe the distribution of dye ions between the bulk solution and the fibre.²⁰⁸

Dyeing is a manifold phenomenon and the precise nature of the dye-fibre interactions still remains a matter of debate. Dye-fibre association may exist in different forms of chemical and physical interactions and more than one type of association may operate at a time.²¹⁰ Dyes and fibres may associate through covalent bonds, ionic bonds, ion-dipole forces, hydrogen bonds, charge-transfer forces, van der Waals forces, hydrophobic interaction and π - π -interaction.^{210,213}

A covalent bond is formed when two participating atoms each contribute one electron to the common linkage and the two electrons are thus shared, uniting the atoms with a bond of high energy. The bonds between reactive dyes and fibre molecules are of this type. Because of the high stability of the covalent bond, such dyes possess high fastness properties against wet treatments.²¹⁰

When positively and negatively charged ions are close to each other, they exert an electrostatic attraction. An ionic bond is formed when an electropositive atom loses an electron to an electronegative atom. Attraction through an ionic bond is important in the dyeing of protein and polyamide fibres.^{210,211}

Ion-dipole forces are formed between a negatively or a positively charged ion, *i.e.*, an anionic or cationic dye, and a polar molecule, *e.g.* water. If in the same molecule, one atom is more electronegative than the other, the bond between these atoms is polar. Polar bonds can cause the molecule to have a substantial dipole moment leading the whole molecule to appear polar. Ion-dipole forces are largely responsible for the aqueous solubility of dyes.²¹⁰

When a hydrogen atom is bonded to an electronegative element through dipole-dipole interaction, a hydrogen bond is formed. Hydrogen bonds can be formed inter- or intramolecularly. Hydrogen bonds are directional and they can be formed only when the two interacting atoms approach very close to each other. Apparently, the distance may be so small that some hydrogen bonds may have a significant amount of covalent or shared electron character.²¹⁴ Thus, a hydrogen bond can be regarded as the result of partial proton donation and acceptance.²¹⁰

So-called charge-transfer complexes are formed when a strongly electron-attracting atom (the acceptor) of one molecule forms a bond with an electron-rich atom (the donor) of another type of molecule. The complexes are characterized as a hybrid of

two resonance structures $[D:A \leftrightarrow D^+ \cdot A^-]$. It is possible that a charge-transfer mechanism is involved in the bonding of disperse dyes to hydrophobic fibres.^{210,215}

Van der Waals forces are usually divided into polarization (or electrostatic) and dispersion (or London) forces. Polarization forces occur when a molecule with a dipole moment is attracted to another molecule by a permanent dipole or by an induced dipole. London forces exist when momentary dipoles of non-polar molecules interact and the motions of their electrons synchronize, causing a continuing electrostatic attraction between them.²¹⁰ Van der Waals forces are sensitive to the distance between the molecules. They are significant only for molecules close to each other, but not too close, because when molecules come too close, the overlap of the electron charge clouds is appreciable and repulsion dominates.^{207,216} Van der Waals attraction depends on the approximate area of contact between two molecules. The greater this area, the greater is the attractive force.²¹⁶ Thus, long dye molecules align as closely and as nearly parallel as possible to the long molecules of a fibre.²¹⁰

Hydrophobic interaction is not actually a new type of bond or intermolecular force. The term is used to describe the tendency of hydrophobic groups, *e.g.* alkyl chains, to associate together and escape from an aqueous environment. Hydrophobic interaction is an effect caused by two forces acting simultaneously: van der Waals forces between the hydrocarbon groups and hydrogen bonds between the water molecules. Each set of forces cause the respective assemblages of molecules or groups to associate together and to exclude the other.²¹⁰ Hydrophobic interactions are important in determining dye uptake by fibres and the wet fastness of dyed material.²¹³

The π - π -interaction is important in dyeing, for example, hydrophobic and hydrophilic fibres with disperse dyes. The aromatic π -electron systems of the fibres may interact with the π -electron systems of the dye molecules. The sorbed dye molecules possess aryl groups that are capable of undergoing π - π -interactions with newly sorbed dye molecules.²¹³

5.1 Mordant dyeing

Chelate formation is as old as the art of dyeing itself and has been utilized with many of the natural colorants, both to give better fixation and, frequently, to give the colour itself. Mordant dyeing is a method, in which the textile is treated with a mordant, *i.e.* tannin or a metal salt. The traditional method is to apply the mordant to the fibre prior to the dye. Alternatively, the metal salt may be added to the dyebath simultaneously with the dye or the mordanting may be accomplished after the actual dyeing process.²¹⁷

Although many transition-metal ions, *e.g.* those of Fe, Cu and Ti salts, are capable of forming complexes with dyes, the metals most widely used as mordants in the dyeing industry are the Cr and Co ions, due to the superior stability of their trivalent complexes.²¹⁸ Traditionally, the amount of dichromate ($K_2Cr_2O_7$) used in mordanting has been 50% of the dye concentration, which refers to the optimal formation of 1:2 complexes. Using the 50% rule, the amount of mordant is usually 0.25–2.5% of the weight of the fibre (owf).²¹⁹ However, this has appeared to be both wasteful and damaging and therefore dye manufacturers have published formulas for calculating reduced levels of dichromate.^{220,221} For example, Ciba-Geigy recommends the following amount:²²¹ $\text{dichromate (\% owf)} = 0.2 + (0.15 \times \text{total amount of dye as a percentage})$. It seems that a dichromate concentration of less than 1% owf has an insignificant influence on the wastewater.²²² Mostly, however, darker shades require larger amounts of mordants.²²² Since chromium compounds are known to be carcinogenic and are thus considered environmentally unfriendly, iron salts could be noteworthy substitutes.²²³ For aluminium mordant, $KAl(SO_4)_2$, an amount of 10% owf is found to be adequate.⁴ The colour hue was not observed to change and the colour-fastness properties were no better when larger amounts of $KAl(SO_4)_2$ mordant were used, but the amount of alum in the wastewater increased.⁴ Thus, it seems that in many traditional and craft dyeing techniques, the amounts of mordants used are excessive.¹ Reduction of the amounts of chemicals to a minimum is highly desirable.

In mordant dyeing, especially in chrome dyeing, the pH of the dye liquor has an effect on the maximum mordanting level. For chrome, the pH range from 3.5 to 3.8 should be attained.²¹⁷ In addition, some electrolytes, such as the sulphate anion of sodium sulphate, have been recognized to inhibit the exhaustion of the dichromate anion by keeping the metal ion in solution in the dyebath. Therefore, avoidance of the use of electrolytes, such as Glauber's salt (Na_2SO_4), is recommended.²¹⁷

The formation of complexes between different metal ions and natural anthraquinone aglycones is explained in Publication IV.

The metal salts used as mordants in dyeing may have a negative effect on the environment. In the biogeochemical cycle, metals accumulate in plants and animals and, by that route, finally also in human beings. Although most metals are important and even necessary for plants and other organisms in trace amounts, they become toxic in high concentrations. In acidic soils, metals remain as soluble cations, in which form they are toxic.^{224,225} The toxicity of the metal ion is influenced by its oxidation state. For example, the Cr(III) ion is less toxic than the Cr(VI) ion. During the dyeing process the Cr(VI) of $K_2Cr_2O_7$ is reduced to Cr(III) in the wool fibre. Chromate salts are listed as human carcinogens and therefore their use has been reduced.²²⁶

5.2 Acid dyeing

Most commercial acid dyes are sodium salts of organic sulphonic or carboxylic acids, where the anion serves as the active colouring component.^{217,227} Acid dyes are used in the coloration of wool, other protein fibres and polyamide. They are applied from dyebaths in acidic or neutral ($\leq$ pH 7.5) conditions.^{217,227} The basic mechanism requires that dye anions are associated with charged NH_3^+ groups in the keratin molecule and that they are bound by electrostatic forces. In addition, other forces such as van der Waals forces, hydrogen bonds, hydrophobic interaction and π - π -interaction may be involved.^{197,213,228}

5.3 Disperse dyeing

Disperse dyes are insoluble in water and they are applied to the fibre from aqueous dispersion. The compact crystalline structure of the synthetic polymer fibres makes the diffusion of disperse dyes into the polymer matrix difficult. In normal disperse dyeing, carriers are often used in order to swell the fibre and aid the penetration and diffusion of the dye. In addition to using carriers, the polymer structure can be loosened by heating the dye liquor to a high temperature (120–130°C) and simultaneously raising the pressure above atmospheric. The high temperature causes a reduction of inter-chain

bonding by thermal agitation of the polymer molecules. The energy of the dye molecules in water increases, thus enhancing dye penetration. Also, the solubility of dyestuffs in water increases slightly.^{229,230} After dyeing, the fibre reverts back to its closely packed stage, thus trapping the dye molecules within the polymer structure.²²⁹ High-temperature disperse dyeing has several advantages compared with normal disperse dyeing: dark shades can be obtained and the process is more economical, as there is no need for a carrier. Thus, expenses can be saved, and also time, because rinsing out of carriers is a lengthy operation.²³¹

6 RESEARCH DESIGN AND PROBLEMS TO BE INVESTIGATED

The main objective of the present study was to investigate the textile dyeing properties of the natural anthraquinone aglycones obtained from the fungus *Dermocybe sanguinea*. This study concerns both traditional craft dyeing and the applications of the pure natural anthraquinone aglycones. The research can be divided into four different parts, which were performed one after the other. The studies began with the fungus and ended up with the colour-fastness properties of the dyed products. The main research problems can be stated as:

1. What kinds of anthraquinone glycoside and anthraquinone aglycone compounds does the fungus *D. sanguinea* contain?
2. How do the pure natural anthraquinone aglycones dye different textile materials as compared with the synthetic dyes, when various dyeing techniques are used?
3. How does the water extract of the air-dried fungus *D. sanguinea*, which contained mainly anthraquinone aglycones, dye natural textile materials, when the amounts of mordants are reduced from those of the traditional procedures and industrial dyeing techniques are applied?
4. What are the colour-fastness properties of textiles dyed with the anthraquinone aglycones of the fungus *D. sanguinea*?

To be able to resolve the main problems, several subsidiary problems had to be dealt with. In answer to the first question, the amounts and characters of the anthraquinone pigments in the fungus *D. sanguinea* were investigated. Isolation of the anthraquinone pigments as aglycones by the new enzymatic method had been accomplished previously by Hynninen. The isolated pigments were analytically separated from each other with 1D and 2D TLC methods. Nokelainen discovered the toluene-ethyl acetate-ethanol-formic acid eluent for the 1D TLC,¹⁷¹ and Hynninen together with Räisänen developed the method further into 2D TLC. The molecular structures of anthraquinones were characterized, using the spectrometric UV/Vis and MS methods. In addition, a preparative multiple liquid-liquid separation technique utilizing stepwise pH gradient was developed by Hynninen and the main pigments were separated on a preparative scale for further dyeing experiments.

To resolve the second question, dyeing tests were made. The pure natural anthraquinones, emodin and dermocybin, were applied as mordant dyes to wool and

polyamide materials, using $\text{KAl}(\text{SO}_4)_2$, $\text{K}_2\text{Cr}_2\text{O}_7$, CoSO_4 , FeSO_4 and CuSO_4 as mordants. Pure emodin and dermocybin were applied as disperse dyes to polyamide and polyester, using the HT-disperse dyeing technique. A mixture of dermorubin and 5-chlorodermorubin was used as an acid dye for wool. In all these experiments, synthetic dyes were used as references and the dyeing properties of the pure natural anthraquinones were compared with the dyeing properties of synthetic dyes.

To obtain an answer to the third research problem, wool and silk materials were dyed in dyebaths containing anthraquinone aglycone mixtures of Fraction 1 and the crude water extract, which also contained mainly anthraquinone aglycones. $\text{KAl}(\text{SO}_4)_2$, $\text{K}_2\text{Cr}_2\text{O}_7$, CoSO_4 , FeSO_4 and CuSO_4 were used as mordants. Because the exact dye concentrations of the crude water extracts in the dyebaths were unknown, these results cannot be compared with those obtained from experiments with the pure compounds.

For the fourth question, the colour-fastness properties of the dyed materials against light, washing and rubbing were tested by the ISO 105-B02, ISO 105-C06 and ISO 105-X12 tests, respectively.^{180–184} The colour-fastness results of the textiles dyed with the pure anthraquinone aglycones were compared with the results of the materials dyed with synthetic dyes. Also, the colour of each dyed material was determined as the CIELAB L^* , a^* and b^* values.

6.1 Methods for isolating, separating and characterizing the pigments of *Dermocybe sanguinea*

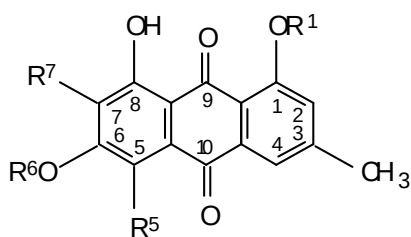
In order to release pigments from their natural cellular environments in fungi or other organisms, the cell structures have to be broken. There are several techniques for cell disruption, for example blending, grinding, osmotic shock and high-pressure extrusion. The method has to be chosen individually by every case taking into account for instance the objective of the experimenter, the particular type of cell and the mass of the material to be processed.²³² In this study, the most convenient method for cell disruption was homogenizing with a very effective homogenizer, Ultra-Turrax. In addition that the homogenizing increases the yield of pigments, it also brings β -glucosidase enzymes and anthraquinone glycosides together so that the enzymatic hydrolysis is possible. Apparently, anthraquinone glycosides are stored in a vacuole, whereas β -glucosidase enzymes are not in the vacuole or associated with the inner tonoplast membrane.¹⁰⁵

Therefore, homogenizing is essential in breaking the cell structures and bringing the enzyme and the substrate together.

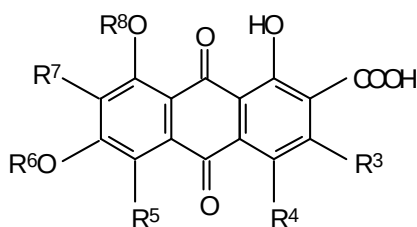
Firstly, the enzymatic method was developed to increase the pigment yield from the fungi. Secondly, it was known that the main pigments in the fungus *D. sanguinea* were emodin- and dermocybin-1- β -D-glucopyranosides.^{129,233} However, the anthraquinone aglycones were presumed to be more suitable for the textile dyeing experiments than the anthraquinone glycosides (*cf.* for example the greater water solubility of anthraquinone glycosides at page 47). The experimental conditions for the isolation of the anthraquinone pigments from the fungus *D. sanguinea*, using enzymatic hydrolysis by the endogenous β -glucosidase, are described in Publication I. Fresh fruit bodies of *D. sanguinea* were used as a starting material. The isolation yielded two fractions: Fraction 1 consisted almost exclusively of anthraquinone aglycones and Fraction 2 consisted mainly of anthraquinone carboxylic acids (Fig. 11). The compositions of the pigments in Fractions 1 and 2 were analysed by 1D TLC combined with densitometric-spectrophotometric measurements. Furthermore, Fraction 1, the mixture of most interest, was analysed more thoroughly by GC-MS after derivatization. The conditions for the 1D TLC and GC-MS are described in Publication I.

Because the 1D TLC yielded unsatisfactory separations, the 2D TLC method was developed. The compounds in Fractions 1 and 2 were separated by the new 2D TLC method and identified by UV/Vis spectroscopy and MS as described in Publication II.

For the textile dyeing experiments, the two main pigments in Fraction 1, emodin and dermocybin, were preparatively separated from each other by a method of multiple liquid-liquid partition (MLLP), using isopropylmethyl ketone/aqueous phosphate buffer as the solvent system and the Hietala apparatus, with 100 partition units, as the partition equipment. The conditions and procedures involved in the experiments are described in Publication III. The partition coefficients obtained from the MLLP showed that even with a few repeated partitions in a separatory funnel, emodin and dermocybin could be obtained in a purity adequate for the dyeing purposes.



Compound	R ¹	R ⁵	R ⁶	R ⁷
Emodin	H	H	H	H
7-Chloroemodin	H	H	H	Cl
5,7-Dichloroemodin	H	Cl	H	Cl
Emodin-1-β-D-Glucp	Glucp	H	H	H
Physcion	H	H	CH ₃	H
Dermocybin	H	OH	CH ₃	OH
Dermocybin-1-β-D-Glucp	Glucp	OH	CH ₃	OH



Compound	R ³	R ⁴	R ⁵	R ⁶	R ⁷	R ⁸
Endocrocin	CH ₃	H	H	H	H	H
5,7-Dichloroendocrocin	CH ₃	H	Cl	H	Cl	H
Dermolutein	CH ₃	H	H	H	H	CH ₃
Dermorubin	CH ₃	OH	H	H	H	CH ₃
5-Chlorodermorubin	CH ₃	OH	Cl	H	H	CH ₃
Austrocorticone	COCH ₃	H	H	CH ₃	H	CH ₃
4-Hydroxyaustrocorticone	COCH ₃	OH	H	CH ₃	H	CH ₃

Figure 11. Chemical structures of the anthraquinones in the fungus *D. sanguinea*. Glucp = glucopyranoside.

6.2 Methods for dyeing with the pure natural and synthetic dyes

The experimental conditions and procedures for the mordant dyeing of wool and polyamide with pure emodin and dermocybin are described in Publication IV. $KAl(SO_4)_2$, $K_2Cr_2O_7$, $CoSO_4$ and $FeSO_4$ were used as mordants in amounts varying from 1 to 10% owf. Wool and polyamide were dyed with emodin in dyebaths of lower pH (pH < 4) and higher pH (~ pH 7–8). The pH of the dermocybin dyebaths was around neutral.^{IV} Acid dyeing of wool with the 2:1 mixture of dermorubin and 5-chlorodermorubin is explained in Publication V. For the HT disperse dyeing of polyester and polyamide with emodin and dermocybin, the experimental conditions and

procedures are described in Publication VI. The pH of the HT-dyebaths was 4.0, because under such conditions emodin and dermocybin were as finely divided dye dispersion, and no additional auxiliaries were needed.^{VI} Maximum dye-uptake measurements, using a spectrophotometer, were performed for each natural anthraquinone in the dyebath at the end of the dyeing process. The method is explained in Publication VI.

Sodium phosphate buffer solutions were used in the experiments to maintain the appropriate pH during the dyeing procedure. In industry, buffer solutions are seldom used, because they make recycling of the dye liquor more difficult. Because the experiments in this research were the first to be carried out on the pure anthraquinone compounds isolated from Nature, they served as a preliminary study and therefore it was necessary to ensure that the appropriate pH was maintained. Experiments were also carried out with different dyebath conditions, *i.e.* with different mordants and buffer solutions.

6.3 Methods for dyeing with anthraquinone mixtures

The aim of studying the dyeing properties of Fraction 1 was to test the possibility of using the anthraquinone mixture as a dye. The procedure by which the anthraquinone aglycones were isolated from the fungus is simple and needs little equipment.^I Thus, the method could easily be utilized by craft workers and others. Textile printing with natural colorants would also benefit from the isolation procedure: in printing the dye must be in a strongly concentrated form preferably as powder in order to obtain opaque print quality.

The dyeing experiments with Fraction 1 were performed in the following way. A 10-g sample of wool fabric was dyed with a 1% of the weight of the fibre (owf) dye solution. A 0.01 or 0.1 M sodium phosphate buffer solution was used to maintain the pH at about 8. To prepare a dyebath, a 100-mg amount of Fraction 1 was dissolved in 200 ml of buffer solution. Then, the Glauber's salt (1% owf) and the mordant were added and the pH was measured (Table III). When ferrous sulphate was the mordant, it was added to the dyebath at the end of the dyeing period as described in Publication IV. A thoroughly wetted fabric sample was immersed in the ready-made dye liquor and the vessel was closed. The temperature was raised from 50°C to 98°C by 2°C/min,

Table III. Conditions in the mordant dyeing experiments when Fraction 1 and the crude water extract of *D. sanguinea* were used as dye liquors. Amounts of the chemicals are described as percentages of the fiber weight (owf). Gls = Glauber's salt. Experiments **1–16** refer to wool and **17** and **18** refer to silk.

Chemicals	Fraction 1			Fraction 1			Crude extract		
	0.01 M Na ₂ HPO ₄			0.1 Na ₂ HPO ₄					
	No.	% owf	pH	No.	% owf	pH	No.	% owf	pH
Buffer									
No Gls, no mordant	1		8.0	8		8.4			
Gls	2	1	8.0	9	1	8.4	14, 17	10	7.0
Gls + KAl(SO ₄) ₂	3	1 + 1	7.4	10	1 + 1	8.1	15, 18	10 + 10	4.5
Gls + K ₂ Cr ₂ O ₇	4a	1 + 1	7.2	11	1 + 1	8.0	16	10 + 1	6.6
	4b	1 + 0.5	7.4						
Gls + CoSO ₄	5	1 + 1	7.4	12	1 + 1	8.2			
Gls + FeSO ₄	6	1 + 1	7.8						
Gls + CuSO ₄	7	1 + 1	7.3	13	1 + 1	8.1			

maintained at 98°C for 1 h and then lowered to 60°C in 10 min. The dyed fabric sample was rinsed with water and dried at room temperature.

Dyeing experiments were performed with the water extract of air-dried *D. sanguinea*, because, in traditional natural dyeing, crude water extracts of plants *etc.* are used as the dye liquors. The author was interested in seeing how the crude water extracts used traditionally would dye fabrics when industrial techniques were applied. The amounts of mordants were also reduced from those recommended in home-dyeing books. The anthraquinone composition in the water extract of air-dried fungi was determined in two different ways utilizing the 2D TLC technique described in Publication II. In the first experiment, 5 g of air-dried fungi were suspended into 100 ml of boiling distilled water, after which the suspension was boiled for 30 min and allowed to cool to room temperature. Suspending into boiling water inactivated the β -glucosidase enzyme. The liquor for the second analytical experiment was performed as described below for the dyebath of the crude extract: a 5-g amount of air-dried fruit bodies were soaked in 100 ml of distilled water overnight, after which the mixture was boiled for 30 min and then allowed to cool to room temperature.

The results from the 2D TLC indicated that the main pigments in the crude water extract dyebath of air-dried *D. sanguinea* were emodin and dermocybin aglycones, and that there were no emodin- and dermocybin-glycosides present in the dyebath. In the

first 2D TLC experiment, where the air-dried fungi were suspended into boiling water, there were emodin- and dermocybin-glycosides present, the ratio between aglycones and glycosides being about 2:1. Dermorubin and 5-chlorodermorubin were observed as the next abundant anthraquinones. Other anthraquinone compounds existed in very small quantities so that either they were observed as traces or not at all on the TLC plate. According to Steglich, 90% of emodin and dermocybin exist as glycosides in the fungus *D. sanguinea*.²³³ However, it is likely that in old fungi the amount of aglycones is higher than in young fungi, because, presumably, ageing disrupts cell membranes and thus enables β -glucosidase enzyme to react with the glycosides. The dried fungi used in this study consisted of both young and old fruit bodies and apparently, less than 90% of emodin and dermocybin existed as glycosides. Nevertheless, it seems that during the drying process part of the *O*-glycosyl linkages in emodin- and dermocybin-1- β -D-glucopyranosides were broken. Possibly, during the evaporation of water the cell membranes were disrupted enabling the β -glucosidase enzymes and anthraquinone-glycosides to meet. Thus, in the first 2D TLC experiment, where the suspending into boiling water inactivated the β -glucosidase enzymes, the result was that emodin and dermocybin were the main pigments, but some amounts of emodin- and dermocybin-1- β -D-glucopyranosides were still present. In the second experiment, during the overnight soaking the β -glucosidase enzymes broke all of the *O*-glycosyl linkages in emodin- and dermocybin-1- β -D-glucopyranosides. Therefore, only anthraquinone aglycones were observed on the TLC plate. However, even though the anthraquinone composition of the crude water extract of the fungus *D. sanguinea* was analysed, it is obvious that there were some amounts of other compounds besides anthraquinones present in the water extract, for example proteins. Furthermore, the dye concentration of the water extract dyebath was unknown and thus, the results of these dyeing experiments are not comparable with those of the dyeing experiments with the pure anthraquinone aglycones.

The chemicals used for these dyeing experiments were the same as those described in Publication IV. The 100% wool yarn was the same as in Publication IV, and was knitted into a tubular fabric and pre-treated as described earlier. The silk fabric was of the tussah type.

The dyebath of the crude extract of the fungus *D. sanguinea* was prepared by soaking a 50 g amount of air-dried fruit bodies in 1.0 l of water overnight, after which the mixture was boiled for 0.5 h, allowed to cool and drained. A 700-ml volume of

dark-red liquor was obtained. Then a 300-ml volume of water was added to the red liquor to obtain a total volume of 1.0 l, which was used for five experiments. A 200-ml volume of the crude extract was measured into a vessel and 1.0 g of Glauber's salt (10% owf) and $\text{KAl}(\text{SO}_4)_2$ (10% owf) or $\text{K}_2\text{Cr}_2\text{O}_7$ (1% owf) mordant were added (Table III). A thoroughly wetted sample of wool or silk fabric was immersed in the dyebath and the vessel was closed. The dyeing conditions for the fabric sample were as described in Publication IV.

6.4 Methods for testing colour fastness of dyed materials

The colour of the dyed material was determined as the CIELAB L^* , a^* and b^* values, using a Chromameter CR-210 equipped with a CIE illuminant C (Minolta, Japan). The measurements were performed on the plain side of the fabric. The fabric was held double layered on a table to avoid the colour of the table from gleaming through. The colour was measured at ten different points on the fabric and the value reported was the mean. Colour fastnesses to light and rubbing were determined according to the ISO 105-B02 and ISO 105-X12 standards, respectively, as described in Publication VI.^{180,182,184} Colour fastness to domestic and commercial laundering was determined according to the ISO 105-C06 standard,^{181,183,184} which is explained in Publication IV for wool and in Publication VI for polyester and polyamide. For polyamide and silk samples, a multi-fibre adjacent fabric was used. The washing-fastness test for silk was performed in a same way as for the wool samples.

7 RESULTS

7.1 Isolation, separation and identification of the anthraquinones from *Dermocybe sanguinea*

Anthraquinone aglycones were successfully isolated from the fungus *D. sanguinea* by a simple and effective enzymatic method, which took advantage of the endogenous β -glucosidase enzyme. A 10.45 kg of fresh fungi yielded *ca.* 60 g (0.6% of the fresh weight of the fungi) of powdered pigments: 56 g of Fraction 1 and 3.3 g of Fraction 2 anthraquinones. Fraction 1 contained almost exclusively the main pigments, emodin and dermocybin, in a ratio of about 2:1, and Fraction 2 contained mainly the anthraquinone carboxylic acids.^I

The main pigments in Fractions 1 and 2 were separated and identified using the 1D TLC combined with densitometric-spectrophotometric measurements. The toluene-ethyl acetate-ethanol-formic acid (10:8:1:2, v/v/v/v) solvent system was found to give relatively good separations. However, both fractions were complex mixtures and several components remained together.^I Therefore, the 2D TLC method was developed.^{II} The *n*-pentanol-pyridine-methanol (6:4:3, v/v/v) solvent system was used as eluent in the first direction and toluene-ethyl acetate-ethanol-formic acid (10:8:1:2, v/v/v/v) in the second direction.^{II} With these solvent systems in 2D TLC, 15 anthraquinone compounds were completely separated from one another. According to the UV/Vis and MS data, five new anthraquinone compounds were found that have not previously been identified in *D. sanguinea*. These compounds were 7-chloroemodin, 5,7-dichloroemodin, 5,7-dichloroendocrocin, austrocorticone and 4-hydroxyaustrocorticone. The other anthraquinones identified were emodin, physcion, endocrocin, dermolutein, dermorubin, 5-chlorodermorubin, emodin-1- β -D-glucopyranoside, dermocybin-1- β -D-glucopyranoside and dermocybin. Due to a very low concentration, one compound remained unidentified. Identification of the compounds is discussed in detail in Publication II. The 2D TLC that was developed provided a simple, fast and effective method for analytical separations of complex anthraquinone mixtures.

The experiments described in Publication III showed that the IMK/aqueous phosphate-buffer solvent system was suitable for the preparative separation of substituted anthraquinones by MLLP. Hydroxyanthraquinones and hydroxyanthraquinone carboxylic acids can easily be resolved from one another on the

basis of the substantial differences in their partition behaviour. In the case of hydroxyanthraquinones, the deviations of the experimental distribution profiles from the theoretical ones were relatively small. Owing to this, the resolution between the most abundant anthraquinones in *D. sanguinea*, dermocybin and emodin was virtually perfect (Fig. 2, Publication III), yielding both compounds in a purity of at least 99%. MLLP in the form of a Martin-Synge Distribution (MSD), was obtained by means of the Hietala apparatus with pH-gradient elution. This method is very suitable for the preparative separation of compounds containing acidic groups such as the phenolic OH and COOH groups, which show great variation in their pK_a values. Because of their strong aggregation properties, these compounds, without derivatization, are very difficult to separate on a preparative scale by chromatographic methods.

7.2 Pure natural anthraquinones as dyes

7.2.1 Mordant dyeing of wool and polyamide with emodin and dermocybin

For wool and polyamide, the uptake values of emodin and dermocybin in the mordant dyeing experiments are listed in Table IV in Appendix 3. The results are discussed in Publication IV. Emodin dyed wool and polyamide yellow in a dyebath with a pH approximately 4 as shown in Colour Figs. 4a and 4b (Appendix 2). However, in a dyebath, where the pH was around 8, the colour was reddish brown (Colour Figs. 5a for wool and 5b for polyamide in Appendix 2). The different colours obtained by using the low and high pH dyebaths were due to the formation of different complexes, *i.e.* octahedral and sandwich complexes, as described in Publication IV. Colour Figs. 6a and 6b (Appendix 2) show the more bluish colour of the wool and polyamide fabrics, respectively, dyed with dermocybin.

The colour of the emodin samples was strongly pH-dependent, as may be clearly seen in Colour Figs. 4 and 5 (Appendix 2). The most significant changes in colour in the washing-fastness tests were obtained for the emodin samples, which were dyed in a dyebath with a low pH. During the washing-fastness test, the yellow colour of the woollen fabric changed to more reddish, because of the basic detergent liquor (Colour Fig. 7 in Appendix 2). Changes in colour also occurred in the corresponding polyamide samples, reddish-brown hues being obtained after washing in alkaline liquor (Colour

Fig. 8 in Appendix 2). In the wool and polyamide, dyed with emodin in a dyebath with a higher pH, some changes were also observed in the colours (Colour Figs. 9 and 10, respectively, in Appendix 2). For the dermocybin-dyed wool and polyamide, only slight changes in colour occurred on washing (Colour Figs. 11 and 12, respectively, in Appendix 2). The pK_a values of dermocybin ($pK_1 = 6.5$, $pK_2 = 10.5$ and $pK_3 = 12.8$ in MeOH^{171}) indicate that the isolated OH group at the position 7 of the anthraquinone ring was ionized in the high pH dyebath. Apparently, the detergent liquor (pH 9) had no additional effect on the dye molecules and therefore no change or only a slight change in colour was observed after washing. The results of rubbing fastness are compiled in Table V. The results in washing fastness and light fastness are listed in Tables III and IV

Table V. Rubbing-fastness properties of wool and polyamide fabrics dyed with pure emodin and dermocybin by the mordant dyeing technique. The numbers in boldface correspond to the dyeing conditions explained in Table I in Publication IV. Dm = direction of manufacture (direction of wales), odm = opposite to the direction of manufacture (direction of courses).

Dye / mordant	WO					PA				
	No.	Dry staining dm	Wet staining odm	Dry staining dm	Wet staining odm	No.	Dry staining dm	Wet staining odm	Dry staining dm	Wet staining odm
Emodin, pH lower:										
No mordant	1	3/4	3/4	1/2	1/2	16	5	5	5	5
KAl(SO ₄) ₂	2	3/4	3/4	1/2	1/2	17	5	5	4/5	4/5
K ₂ Cr ₂ O ₇	3	3	3	2	2					
CoSO ₄	4	3/4	3/4	1/2	1/2	18	5	5	5	5
FeSO ₄	5	4	4	2/3	2/3	19	5	5	5	5
Emodin, pH higher:										
No mordant	6	4/5	4/5	3	3	20	4/5	4/5	4/5	4/5
KAl(SO ₄) ₂	7	4	4	3	3	21	4/5	4/5	4/5	4/5
K ₂ Cr ₂ O ₇	8	4/5	4/5	2/3	2/3					
CoSO ₄	9	4/5	4/5	3	3	22	4	4	4/5	4/5
FeSO ₄	10	4/5	4/5	3	3	23	5	4/5	4/5	4/5
Dermocybin:										
No mordant	11	2	2	1/2	1/2	24	4/5	4/5	4/5	4/5
KAl(SO ₄) ₂	12	1/2	1/2	1/2	1/2	25	3	3	3/4	3/4
K ₂ Cr ₂ O ₇	13	2	2	1/2	1/2					
CoSO ₄	14	2/3	2/3	1/2	1/2	26	2/3	2/3	2/3	2/3
FeSO ₄	15	1/2	1/2	1	1	27	2	2	2/3	2/3

of Publication IV, respectively. The degree of colour fastness of wool and polyamide fabrics dyed with emodin and dermocybin are explained and discussed in detail in Publication IV.

The washing-fastness tests confirmed that the natural dyes show changes in hue when treated with a weakly alkaline solution.¹⁹¹ In textiles, the colour change produced by washing is undesired. However, such dyes could be used, for example, in products of art handicraft that need no washing.

7.2.2 Acid dyeing of wool with dermorubin and 5-chlorodermorubin

The experiment using a mixture of anthraquinone carboxylic acids, dermorubin and 5-chlorodermorubin, as acid dyes for wool gave good results. Dermorubin and 5-chlorodermorubin dyed wool orange-red with good colour-fastness properties. The results indicated that wool had a high uptake for the anthraquinone carboxylic acids. Apparently, the ionic bonds between the carboxylate ions of the dye and the protonated amino groups of the wool enhanced the colour fastness of the dyed fabric. The actual colours of wool fabrics dyed with the natural and synthetic acid dyes can be seen in Colour Fig. 13 (Appendix 2). The results of acid dyeing experiments are discussed in detail in Publication V.

7.2.3 High-temperature disperse dyeing of polyester and polyamide with emodin and dermocybin

The HT disperse dyeing technique gave excellent results for polyester.^{VI} Emodin and dermocybin dyed polyester brilliant yellow and orange with very good colour fastnesses against light, washing and rubbing. The fastness properties of polyamide dyed with emodin and dermocybin were not as good as those of polyester. Dermocybin received poor value in the light fastness test, and the colour change value of emodin in washing fastness test was low. The results of HT disperse dyeing of polyester and polyamide are explained and discussed in Publication VI. Colour Figs. 14 and 15 (Appendix 2) show the colours of polyester and polyamide fabrics dyed with emodin and dermocybin, respectively. It will be noticed that the colours of polyester and polyamide materials,

though dyed with the same dye, differ significantly. Apparently, the dye aggregates are bound to these materials in different ways. Delusterants, optical brighteners and other fibre pre-treatments may also have affected the colour.

As stated before, a colour change after washing is characteristic of materials dyed with the natural anthraquinone aglycones. However, for disperse-dyed polyester no such change in colour was observed. In the HT disperse dyeing technique, the anthraquinone aggregates were trapped inside the polymer structure. Thus, the dye molecules were not in contact with the alkaline washing liquor and deprotonation of the OH groups did not occur.^{VI}

7.3 Natural anthraquinone mixtures as dyes

Table VI summarizes the CIELAB L*, a* and b* values for the wool samples dyed with Fraction 1 and the colours obtained are shown in Colour Fig. 16 (Appendix 2). It may be noticed from the CIELAB values of samples **1** and **2**, as well as **8** and **9**, that Glauber's salt does not affect the colour. In a dyebath, it works only as an electrolyte. The KAl(SO₄)₂ mordant brightens the hue, as is indicated by the slightly higher L* value of samples **3** and **10**. The chrome mordant (K₂Cr₂O₇) alters the hue towards blue, which can be seen from the low b* values of samples **4a**, **4b** and **11**. When the stronger 0.1 M sodium buffer solution is used, the colour is even more strongly bluish than when the 0.01 M buffer is used, as is indicated by the negative b* value of sample **11**. The low b* values and the slight reduction in the a* values of samples **4a** and **4b** indicate a colour change towards blue. Apparently, the cobalt mordant gives the fabric a brown colour. Iron mordant turns the hue green, as is indicated by the relatively low values of a* and b* in sample **6**.

The dye-uptake measurements of Fraction 1 (Table VII in Appendix 4) indicate that, when a 0.01 M buffer solution was used, the uptake of anthraquinones varied from 68 to 93%, being above 80% when mordants were used. But, when 0.1 M buffer solution was used, the uptake was lower and varied from 60 to 93%, being in most cases around 60%. Dye uptake reached 93% only when K₂Cr₂O₇ was used as mordant. Apparently, the use of mordants increased the dye uptake. In the other cases, however, the strong buffer solution seemed to inhibit the dye uptake although, not when chrome was used as the mordant. The positive and negative anions of the buffer kept the metal

ions as well as the dye ions in solution. There was an insignificant difference in the dye uptake or the colour between experiments **4a** and **4b** (Tables III and VI, Table VII in Appendix 4, Colour Fig. 16 in Appendix 2). Thus, a smaller amount of the $K_2Cr_2O_7$ mordant seems to be adequate for dyeing.

The colour-fastness properties of the wool fabrics dyed with Fraction 1, using different mordants, are listed in Table VIII. The $KAl(SO_4)_2$ mordant seems to decrease the light fastness. Chrome-mordanted samples gave the best results for colour fastness and there was hardly any difference between the results of samples **4a** and **4b**. When $CuSO_4$ (**7**, **13**) and $CoSO_4$ (**5**, **12**) were used as mordants, slightly better results were obtained than when $KAl(SO_4)_2$ was used as a mordant (**3**, **10**).

Table VI. The CIELAB L^* , a^* and b^* values for wool dyed with Fraction 1, and the ΔE , ΔL , Δa^* and Δb^* values of the dyed and washed fabric as compared with the dyed unwashed fabric. The CIELAB parameters are explained in Chapter 2.2.3. GlS = Glauber's salt. The numbers in boldface correspond to the dye bath conditions explained in Table III. The corresponding colours are shown in Colour Fig. 16 (Appendix 2).

No.	Fraction 1	L^*	a^*	b^*	ΔE	ΔL	Δa^*	Δb^*
0.01 M Na_2HPO_4 :								
1	No GlS, no mordant	37.83	+28.30	+15.87	1.87	+0.77	+0.69	-1.57
2	No mordant	35.20	+30.29	+15.08	1.92	+1.42	+0.51	-1.19
3	$KAl(SO_4)_2$	42.74	+33.90	+26.29	2.10	+0.91	+0.94	-1.65
4a	$K_2Cr_2O_7$ (1%)	25.12	+22.35	+1.16	0.72	+0.71	-0.01	-0.14
4b	$K_2Cr_2O_7$ (0.5%)	24.49	+21.95	+2.92	0.85	+0.70	-0.13	-0.48
5	$CoSO_4$	30.52	+21.79	+10.03	2.53	+2.36	+0.77	+0.51
6	$FeSO_4$	38.01	+8.58	+15.27	3.08	+1.28	+1.87	-2.09
7	$CuSO_4$	46.93	+14.08	+23.31	2.29	+1.20	+0.28	-1.94
0.1 M Na_2HPO_4 :								
8	No GlS, no mordant	33.35	+31.49	+11.47	1.96	+1.49	+0.85	-0.96
9	No mordant	33.35	+30.74	+11.03	1.98	+1.68	+0.63	-0.84
10	$KAl(SO_4)_2$	40.21	+35.38	+21.00	1.73	+1.52	+0.35	-0.77
11	$K_2Cr_2O_7$	20.95	+15.23	-2.60	0.92	+0.90	+0.11	+0.19
12	$CoSO_4$	30.64	+19.65	+8.00	2.89	+2.60	+0.58	+1.13
13	$CuSO_4$	44.20	+14.74	+20.67	1.92	+1.48	-0.21	-1.22

Table VIII. Colour-fastness properties of the wool fabrics dyed with Fraction 1. The washing-fastness results in colour changes are given in two ways: as a grey-scale rating converted from ΔE values⁸⁸ (Table VI) and as a visually assessed grey-scale rating. LF = light fastness, vis ass = visual assessment, dm = direction of manufacture (direction of wales), odm = opposite to the direction of manufacture (direction of courses), Gls = Glauber's salt, bl = bluer. * = the first value was obtained according to the ISO 105-A04 standard and the second value was obtained by visual assessment. For light fastness, the rating values vary in the range 1–8, 8 being the best value.¹⁸⁰ The numerical rating for colour change is given by the grey-grey scale numbers 1–5, 5 being the best value.^{181,183} The numerical rating for staining is given by the gray-white scale numbers 1–5, 5 being the best value.^{181,182,184}

No.	Fraction 1 / Chemicals	LF	Washing fastness		Staining				Rubbing fastness			
			Colour change from ΔE	vis ass	WO*		CO*		Dry staining		Wet staining	
					dm	odm	dm	odm	dm	odm	dm	odm
0.01 M Na ₂ HPO ₄ :												
1	No Gls, no mordant	3	4	2, bl	4	3	3/4	3	2/3	2/3	2/3	2/3
2	Gls	3	4	2, bl	4	3	3/4	3	2/3	2/3	3	2/3
3	Gls + KAl(SO ₄) ₂	2	3–4	2, bl	4/5	3	4	3	1	1	1/2	1/2
4a	Gls + K ₂ Cr ₂ O ₇ (1%)	5	4–5	3, bl	4/5	4/5	4/5	4	3	3	3	3
4b	Gls + K ₂ Cr ₂ O ₇ (0.5%)	5	4–5	3, bl	4/5	4	4	4	3	3	2/3	2/3
5	Gls + CoSO ₄	4	3–4	1, bl	4	3/4	4	3	1/2	1/2	2	2
6	Gls + FeSO ₄	4	3	2, bl	3/4	3	3/4	3	1	1	1/2	1/2
7	Gls + CuSO ₄	5	3–4	3, bl	4/5	4/5	4/5	4	1/2	1/2	2	2
0.1 M Na ₂ HPO ₄ :												
8	No Gls, no mordant	3	4	1, bl	3/4	2/3	3/4	2/3	3/4	3/4	3	2/3
9	Gls	3	4	1, bl	3/4	2/3	3/4	2/3	3/4	3/4	3	3/4
10	Gls + KAl(SO ₄) ₂	2	4	2, bl	3/4	3	3/4	3	1/2	1/2	1/2	1/2
11	Gls + K ₂ Cr ₂ O ₇	6	4–5	4	4/5	4/5	4/5	4/5	4	4	2/3	2/3
12	Gls + CoSO ₄	5	3–4	1	4	3/4	3/4	3	2/3	2/3	2	2
13	Gls + CuSO ₄	5	4	2, bl	4/5	4	4	3/4	2/3	2/3	2	2

The colour changes as a result of washing are given in two ways: as a grey-scale rating converted from the ΔE values (Table VIII) and as a visually assessed grey-scale rating.⁸⁸ These two values differ considerably from each other (Table VIII). As stated before in Chapter 3, the difficulty of visual assessment is obvious. The values obtained from instrumental assessment may be considered more reliable. The instrumentally and visually assessed values for staining do not seem to vary greatly (Table VIII). The colour differences between the unwashed and washed fabrics are shown in Colour Fig. 17 (Appendix 2).

The wool and silk fabrics dyed with the anthraquinone aglycones in the crude water extract of air-dried *D. sanguinea* are shown in Colour Figs. 18a and 18b (Appendix 2), and the corresponding CIELAB L*, a* and b* values are listed in Table IX. It will be noticed, on comparing the wool samples in Colour Figs. 16 and 18a (Appendix 2), that the hues of corresponding samples are very similar. The darker shades in Colour Fig. 18a (Appendix 2) indicate that the concentration of dyestuff in the crude extract dyebath was much higher than 1% (owf). In addition, the dark colour of the dyebath after the dyeing indicated that it still contained a great deal of dye. In the case of the 1% dyebath of Fraction 1, the liquor was nearly colourless after the dyeing process. The resembling colours in Colour Figs. 16 and 18a (Appendix 2) confirm that Fraction 1 and the crude water extract contained anthraquinone aglycones emodin and dermocybin as the main colorants.

For the wool dyed with the crude extract of *D. sanguinea*, from low to moderate colour-fastness properties were obtained even without mordant. The very high concentration of anthraquinones in the crude extract influenced the results, for example, by increasing the light fastness but decreasing the rubbing fastness. The rubbing-fastness results for wool were very low due to the great amount of loosely fixed dye on the surface of the fibre (Table X). Because of the level and hard surface of the silk fabric, the rubbing-fastness results were better than those for the wool fabric (Table X).

Table IX. The CIELAB L*, a* and b* values for wool and silk dyed with the crude extract of *D. sanguinea*, and the ΔE , ΔL , Δa^* and Δb^* values of the dyed and washed fabric as compared with the dyed unwashed fabric. The numbers in boldface correspond to the dye bath conditions explained in Table III. Experiments **14–16** refer to wool and **17** and **18** refer to silk. The corresponding colours are shown in Colour Figs. 18a and 18b (Appendix 2).

No.	Fibre / Mordant	L*	a*	b*	ΔE	ΔL	Δa^*	Δb^*
14	WO / No mordant	24.27	+26.51	+11.01	1.01	+0.72	+0.54	-0.46
15	KAl(SO ₄) ₂	35.40	+32.43	+28.30	2.02	-0.38	-0.29	-1.97
16	K ₂ Cr ₂ O ₇	17.62	+12.85	-0.08	0.43	+0.37	+0.22	-0.03
17	SE / No mordant	44.14	+29.20	+15.65	4.47	+3.99	-0.02	-2.02
18	KAl(SO ₄) ₂	48.77	+33.40	+31.81	3.58	+1.86	+1.44	-2.70

Table X. Colour-fastness properties of wool and silk dyed with the crude extract of *D. sanguinea*. The washing-fastness results in colour changes are given in two ways: as a grey-scale rating converted from ΔE values⁸⁸ (Table IX) and as a visually assessed grey-scale rating. LF = light fastness, vis ass = visual assessment, r = redder, bl = bluer, dm = direction of manufacture (direction of wales and warps), odm = opposite to the direction of manufacture (direction of courses and wefts). * = the first value was obtained according to the ISO 105-A04 standard and the second value was obtained by visual assessment. For light fastness, the rating values vary in the range 1–8, 8 being the best value.¹⁸⁰ The numerical rating for colour change is given by the grey-greyscale numbers 1–5, 5 being the best value.^{181,183} The numerical rating for staining is given by the grey-white scale numbers 1–5, 5 being the best value.^{181,182,184}

No.	Fabric / mordant	LF	Washing fastness		Staining						Rubbing fastness			
			Color change from ΔE	vis ass							Dry staining		Wet staining	
											dm	odm	dm	odm
	WO /				WO*			CO*						
14	No mordant	5	4–5	2	3	2		3	2/3		1	1	1	1
15	KAl(SO ₄) ₂	5	4	3, r	3/4	3/4		3/4	3/4		1/2	1/2	1/2	1/2
16	K ₂ Cr ₂ O ₇	6	4–5	4	4/5	4/5		4	4/5		2	2	1/2	1/2
	SE /				WO	CA	SE	PA	CO	CTA				
17	No mordant	2	2–3	2, bl	4	4	4	4	3/4	4	4	3/4	3	3
18	KAl(SO ₄) ₂	3	3	2–3, r	4	4/5	4/5	4	4	4	4	4	3/4	3/4

8 DISCUSSION

8.1 Reliability of the dyeing results

The aim of this study was to investigate the use of natural anthraquinone aglycones from the fungus *D. sanguinea* as dyes for textile materials. The author was interested in both the industrial dyeing techniques and the traditional craft dyeing techniques. Natural fibres, wool and silk, and synthetic fibres, polyamide and polyester, were coloured using mordant dyeing, acid dyeing, and HT disperse-dyeing techniques. Different dyes (*i.e.* emodin, dermocybin, dermorubin and 5-chlorodermorubin, Fraction 1 and the water extract of air-dried fungi), different dyeing techniques, and different fabrics were used, so as to explore the dyeing properties of the natural anthraquinone aglycones as widely as possible. The colour fastness of the dyed materials was tested according to the ISO standards. For the normal use of a textile, light-fastness and washing-fastness tests are the most important. However, rubbing-fastness tests were also performed. The rubbing-fastness test gave information about the fixation of the dye aggregates to the surface of the fabric.

The reliability of a study reflects the consistency, repeatability, and predictability of the results. High reliability may be obtained by accurate measurements, which eliminate random results. Because the colour-fastness tests were evaluated subjectively, *i.e.* visually, there may have been some variation in the reliability of the test results. Greater reliability would have been obtained, if the colour-fastness tests had been evaluated several times and if more than one assessor had been involved. On the other hand, according to the literature, the colour-fastness values between different assessors, and even between repeated assessments by one and the same assessor, may vary by as much as three points.^{185,186} Thus, to diminish deviations, samples would have had to be evaluated some twenty times, which would have increased the work tremendously. Instrumental assessment methods were used when ever possible. The colour changes in the washing-fastness tests were evaluated both subjectively and objectively. The washing fastness of staining was also tested subjectively and objectively in the wool and polyester samples. With regard to the polyamide and silk samples, a multifibre test material was used, which could not be measured with the CIELAB instrument, because the area of each fibre was too small. When comparing the subjective and objective colour changes and staining values of washing fastness, it was discovered that the

differences between subjective and objective values were greater for colour change than for staining. It seems that it was more difficult to evaluate the change of colour visually, using the grey-grey scale, than to evaluate the staining of the test material using the grey-white scale. On the other hand, the objective value, which was converted from the instrumentally measured ΔE value, took into account the total change in colour in terms of the L^* , a^* and b^* parameters. Hence, the grey-scale value for colour change converted from ΔE was low. In contrast, visually, no significant fading of the colour was observed, only the change in hue. Thus, the visually assessed grey-scale value was high.

8.2 Environmental perspectives

Over the past decades, concern for the environment has gained increasing importance and regulations have been established to protect both the environment and human health. The overall goal of sustainable development demands, among other objectives, a substantial reduction in the consumption of raw materials and energy.²³⁴ Industry has had to meet the requirements of this legislation by reducing the amounts of resources consumed, the amount and hazard of waste and of emissions that are generated, as well as the risk of accidents and malfunction.²³⁴ This has led to close analysing and optimising of processes. For example, the procedures for the production of synthetic dyes have had to be optimized in respect of high yield and especially of purity, because purification processes after synthesis would be too expensive for large-scale operations.⁷¹ Because of imperfect synthesis, some commercial dyes may contain impurities amounting to high percentages and, moreover, some of these impurities may be toxic.²³⁵

Low labour costs and evidently also environmental costs are major incentives for companies to change location. The textile and dye industries have already shifted eastward. Without international co-operation, there is a threat that dyes which are banned in western countries, *e.g.* benzidine-based dyes, are being used in the Far East, from where they still may end up in western markets. Without global co-operation, the end-result will merely be the relocation of sources of pollution rather than their reduction.²³⁶

The main pollution characteristics of the wastewater of the textile industry were noted as intense colour, high pH and alkalinity, high chemical oxygen demand, high chlorine content, high temperature, and toxicity of the sulphur in dye wastes.²³⁷ Nowadays, the major environmental problem in the textile dyeing industry is the removal of dyes from effluents. The dye concentration may be well below 1 ppm (0.001 mg/l), which is lower than that of many other chemicals found in wastewater, but the dye is visible even at very low concentrations, thus producing an aesthetic hazard.^{69,71}

Several methods for treating wastewater have been developed and many of them have been studied intensively to obtain better results. Methods for decolorization of wastewater are adsorption, precipitation, chemical degradation, photo-degradation and biodegradation.⁷¹ Charged particles such as dye and metal ions can be removed by electrochemical methods.²³⁸ Adsorption is an effective method for lowering the concentration of dissolved organic matter in an effluent. The adsorbent most widely used for dyes is carbon.²³⁹ In one method, azo dyes are chemically reduced with sodium borohydride to smaller amines, which are colourless. These amines are further adsorbed on carbon, oxidized, or treated biologically.²⁴⁰ Of the chemical processes, the most important in practice is oxidative degradation by chlorine or by ozone.⁷¹ Ozone (O₃) attacks the most reactive site in the dye molecule, *i.e.* the carbon-carbon double bond, leading to decomposition of the molecule. Ozonation alone is often insufficient, however, and it is then necessary to combine the ozone treatment with, for example, carbon or active sludge treatments.²⁴¹ The disadvantage of physico-chemical removal of dyes from effluents is the high cost and, although the dyes are removed, accumulation of the concentrated sludge creates a disposal problem.

Biodegradation to complete mineralization would be an ideal method for decolourizing effluents. However, synthetic dyes are xenobiotic. The natural systems of micro-organisms seldom contain enzymes that degrade such compounds under aerobic conditions, and even under anaerobic conditions in sewage sludge, degradation of dyestuffs takes place very slowly.⁷¹ However, biodegradation for decolourizing wastewater has been studied intensively in recent years. According to a recent article,²⁴² application of solid-state fermentation (SSF) with white-rot fungi, offers a means of riddance of dye-containing effluents. SSF of the waste with adsorbed dye will degrade the dye and simultaneously enrich the nutritional value of the waste, which can then be used as a soil conditioner or fertiliser.²⁴²

Dye wastewaters are extremely variable in composition, on account of the large number of dyes and other chemicals used in dyeing processes. As toxicity standards become more widespread and more stringent, the development of technological systems for minimizing the concentration of dyes and their breakdown products in wastewater will become necessary. Apparently, the complexity of textile dye wastewater precludes a universal treatment system, suitable for all textile facilities. Thus, to obtain the best results, treatment systems must be evaluated individually case by case.²⁴³

Fashion has a role to play in determining the nature of textile effluents, because it affects the fabrics and colours that are used. Also, dark shades increase the amount of dye in effluents. Modern textiles are required to have high degrees of chemical and photolytic stability in order to maintain their structure and colour. In addition, anti-microbial agents are sometimes added, particularly to natural fibres, to make them resistant to biological degradation. The high colour-fastness, stability and resistance of dyes to degradation have made removal of colour from textile wastewaters difficult.⁶⁹

The degree of fixation of a dye to a fibre plays an important role in reducing dyes in effluents. Disperse and metal-complex dyes, and also basic dyes, have the highest degrees of fixation, *i.e.* around 90–100%.⁶⁹ The reactive dyes, which are mostly used in dyeing cotton, present the greatest problem. The degree of fixation for reactive dyes on cotton varies from 50 to 90%. The problem is obvious, because of the dominance of cotton in the fashion industry.⁶⁹

Increasing environmental awareness has stimulated textile firms to define eco labels and trademarks, which set environmental criteria for textile products. The criteria for different eco labels are based on three approaches.^{244,245} The first approach is based on a series of relevant tests of the finished product and may be considered a guarantee of consumer health and safety. The second approach is based on environmental auditing and analysis of the products and processes. It is thus closer both to industry and to the environment than the other two approaches. The third approach, which is the most complete, is based on a lifecycle analysis of the product. Even though eco labels give mainly environmental criteria for products, the labels may be considered as marks of quality.^{245,246}

8.3 Further aspects of natural dyes

This study was based on the multidisciplinary approach of using natural colorants as textile dyes. It started from the fungi and resulted in the dyed products. Many interesting results were obtained and it is to be hoped that at least some of them will find applications in the future. This research served as a pilot study for the application of pure natural anthraquinone aglycones as textile dyes. Of the many interesting subjects for further research, the following would be the most interesting.

The study of the anthraquinone metal complexes formed in mordant dyeing was beyond the scope of this study. However, it would be interesting to investigate the formation of emodin and dermocybin complexes with various metal atoms. Especially, the possible formation of a complex with a repeated dermocybin metal sequence would be worth investigating.^{IV}

The HT-disperse dyeing experiments, especially on polyester, had the best results in colour fastnesses. Thus, further experiments with other synthetic fibres, for example with polyethylene and polypropylene, would be interesting.

Emodin is probably the most widely distributed anthraquinone, being present in moulds, higher fungi, lichens, flowering plants and insects.¹⁰⁶ For pharmaceutical studies, emodin has been obtained from fungi, which have been cultivated on a culture medium.²⁴⁷ *Penicillium clavariaeformis* Solms was used in the experiments, but some *Dermocybe* and *Cortinarius* species were mentioned as worth investigation.²⁴⁷ Possibly, in the future anthraquinone dyes could also be produced by cultivating fungi and extracting the pigments, which could then be modified further by chemical means. Even though *Dermocybe* is a mycorrhizal fungus and so difficult to cultivate,¹⁴⁶ it would be interesting to make such experiments. The ectomycorrhizal fungus of *Cantharellus cibarius* has been successfully cultivated and it would be interesting to try similar methods with *Dermocybe*.^{248–250}

From the ecological point of view, fungi serve as an interesting source of raw material. In addition to *Dermocybe* species, some other fungi are known to contain strong colouring components. For instance, it is known that *Telephora palmata*, *Sarcodon imbricatus*, *Hydnellum suaveolans* and *Hapalopilus rutilans* impart a blue colour to wool fibres.^{50–52,54} Because blue colours are rare in nature, the pigments in these fungi would be of great value for textile dyeing.

Green pigments in plants and algae form a vast source of potential colouring agents.¹⁷⁴ It is known that many plants give a green colour when their water extracts are used for dyeing textiles. There have been several studies, in which crude extracts from green leaves have been used as dyes for hair and textile fibres.^{251–254} For some time already, chlorophyll derivatives, such as copper chlorophyllins, have been used as colorants in foods, cosmetics and pharmaceuticals.^{255–257} Apparently, there have been no studies in which pure chlorophyll derivatives have been used as dyes for textiles. However, they would seem to offer opportunity for closer research.

8.4 Natural dyes in teaching

Natural dyeing offers a theme for the integration of biology, chemistry and craft lessons at high-school level. Integration between different subjects is desirable, since it enhances the student's understanding of complete processes. The curriculum of natural dyeing would contain the whole process from the fungus to the finished craft products. The process would include identification and picking of the fungus, isolation and identification of the anthraquinone pigments, dyeing with isolated powdered pigment and making the dyed material into the finished products.

In the curriculum, biology classes would be used for the collection and identification of the fungus. This part could be included in the teaching, when forest biology and ecology are studied. Apparently, the *Dermocybe* forms mycorrhizae with spruce, birch and willows. The symbiosis between trees and fungi could be explained and demonstrated.

In chemistry classes, the colourful anthraquinone pigments in *Dermocybe* would be studied. For example, in the seventh grade chemistry book (in the Finnish comprehensive school), basic laboratory procedures, such as extraction, distillation and chromatography, are explained. In addition, most schools are equipped with hoods which are essential for the safety. The isolation procedure for the pigments from the fungi, utilizing the endogenous β -glucosidase and the analytical 2D TLC method developed in the current study, are relatively simple techniques being feasible at school. The handling and distillation of acetone, a flammable organic solvent, in the isolation process requires carefulness and is the most demanding stage of the procedure. The anthraquinones would be separated and analyzed utilizing the 2D TLC technique. The

R_f -values of each compound would be calculated and the compounds would be identified by comparing the R_f -values and the observed colours of the spots with those mentioned in the literature. Some chemical and physical aspects of the dyeing process could also be discussed in chemistry lessons.

In crafts, the anthraquinone pigment powder of Fractions 1 and 2 would be used for dyeing or printing of wool and silk materials. Dyed or printed materials would be made into products, for example socks, gloves, sweaters, embroidery or scarves.

9 CONCLUSIONS

A simple method for the isolation of hydroxyanthraquinone aglycones from fungi was developed in this study.^I This is useful in natural dyeing and printing, as the concentration of dyestuff can easily be measured and controlled, because the colouring components can be handled as a powder. As a result of the isolation procedure, two fractions were obtained: one consisting mainly of emodin and dermocybin and the other consisting mainly of the anthraquinone carboxylic acids. The main pigments emodin and dermocybin, in a ratio of about 2:1, formed 94% of the total amount of pigment.

The 2D TLC technique developed proved to separate complex mixtures of substituted anthraquinones; fifteen different anthraquinones being completely separated from one another. The excellent separation power of the new 2D TLC technique resulted in finding five compounds not previously identified in the fungus *D. sanguinea* in which they existed in very low concentrations.^{II} The five new compounds were 5-chloroemodin, 5,7-dichloroemodin, 5,7-dichloroendocrocin, 4-hydroxyaustrocorticone and austrocorticone. The other identified compounds were emodin, physcion, endocrocin, dermolutein, dermorubin, 5-chlorodermorubin, emodin-1- β -D-glucopyranoside, dermocybin-1- β -D-glucopyranoside and dermocybin.

The MLLP offered an excellent method for the preparative separation of the compounds, which contain acidic groups such as the phenolic OH and COOH groups.^{III} On account of their strong tendency to aggregate, these compounds are, without derivatization, very difficult to separate on a preparative scale by chromatographic methods.

The dyeing experiments showed that the natural anthraquinone aglycones had relatively high affinities with textile fibres. The pure anthraquinone compounds emodin and dermocybin, as well as dermorubin and 5-chlorodermorubin, dyed natural and synthetic fibres successfully. The colour fastness of the dyed materials varied from low to excellent among the different fibres and different dyeing techniques. Excellent results were obtained especially in the case of HT-dyed polyester. In the HT experiments, practically all of emodin and dermocybin dyes were absorbed into the fibres. As a result of the mordant dyeing experiments, it seems reasonable to reduce the amounts of mordants recommended in the traditional dyeing recipes, because adequate colour-fastness properties were obtained using 10% owf of alum and 1% owf of other mordants. However, when taking into account the environmental and health aspects, it

should be considered what mordants would be reasonable to use. Even though moderate colour-fastness properties were obtained without using any mordant, it is obvious that, to ensure the highest possible quality of dyed products, some mordants are needed. According to this study, natural anthraquinone aglycone mixtures dyed wool and silk fibres well in mechanical dyeing processes even at low concentrations of the mordants, *i.e.* when 10% owf of $\text{KAl}(\text{SO}_4)_2$ and 1 or 0.5% owf of the other mordants were used.

As a result of the study, it can be stated that naturally occurring anthraquinone pigments offer significant opportunities for dyeing textiles. It is known that the total syntheses of substituted anthraquinones are relatively cumbersome, frequently requiring toxic reagents, such as chromic acid or mercuric salts, and producing considerable amounts of toxic waste chemicals. To avoid the disadvantages or deleterious effects of heavy synthetic processes, one should realize that Nature is often the best synthesizer. In the biosynthetic processes enzymes act as catalysts, and no toxic side products or wastes are produced. Nature is rich in pigments and one only has to discover a suitable technique for the isolation of the pigments from the natural source in concern. The pure natural compounds can be used as pigments straightforwardly or they can be used as raw material and modified further by chemical means to change their properties into the desired direction. In this study, the HT disperse-dyeing experiments with emodin and dermocybin gave the best results: the dye uptake was nearly 100%, the colour-fastness properties were excellent, and the dyeing procedure did not require any auxiliary substances. In addition, the colour of the dyed material did not change on washing. As yet, no syntheses have been proposed for dermocybin and, thus, its isolation from nature is the only way of obtaining the pigment.

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APPENDIX 1. Author's contribution to the research represented in the publications.

Publication I (P. H. Hynninen, R. Räisänen, P. Elovaara and E. Nokelainen, 2000):

- Writing the manuscript.
- Interpretation of the 1D TLC chromatograms and densitograms.
- Derivatisation of Fraction 1 for GC-MS. Preliminary GC analysis of the compounds by operating the instrument and interpreting the GC chromatograms.
- Interpretation of the GC-MS data and suggestion for the formation of the sandwich dimer in IMK solution.

Publication II (R. Räisänen, H. Björk and P. H. Hynninen, 2000):

- Writing the manuscript.
- Development of the 2D TLC method together with Hynninen.
- Interpretation of the 2D TLC chromatograms.
- Separation of the anthraquinones for the UV/Vis and MS measurements. Running the UV/Vis spectra. Interpretation of the UV/Vis and MS data.

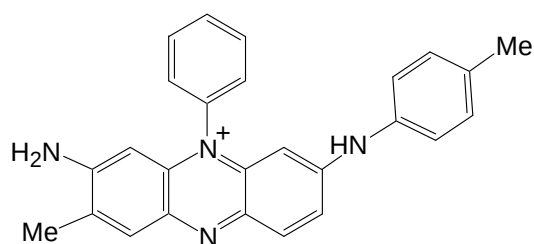
Publication III (P. H. Hynninen and R. Räisänen, 2001):

- Writing the manuscript together with Professor P. H. Hynninen.
- Calculation of the theoretical distribution profiles and the partition coefficients, k , for the compounds.
- Interpretation of the MLLP elution diagrams using TLC analysis.

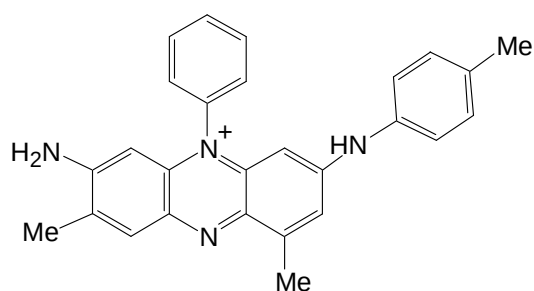
Publications IV–VI (R. Räisänen, P. Nousiainen and P. H. Hynninen, 2001 and in print):

- Writing the manuscripts.
- Designing and accomplishing the dyeing experiments.
- Performing the colour-fastness tests.
- Interpretation of the results.

APPENDIX 2. Colour Figures.



The major component of Mauveine



The minor component of Mauveine

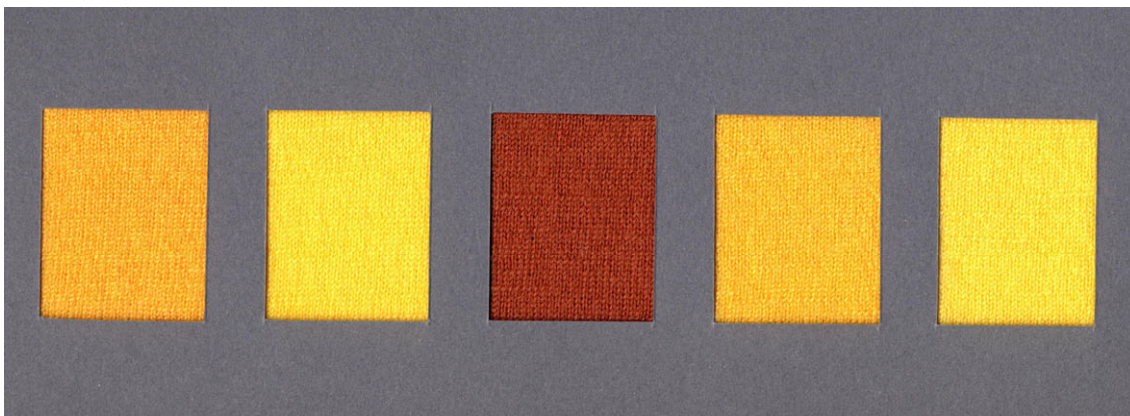
Colour Figure 1. Some yarn dyed with Mauveine and with another important dye from the 19th century, alizarin.⁷⁶ Perkin's Mauveine is actually a mixture of two major compounds with the structures shown above.⁵⁹ (For the colour figure, copyright © 1983 Kurt Nassau. Reprinted by permission of John Wiley & Sons).



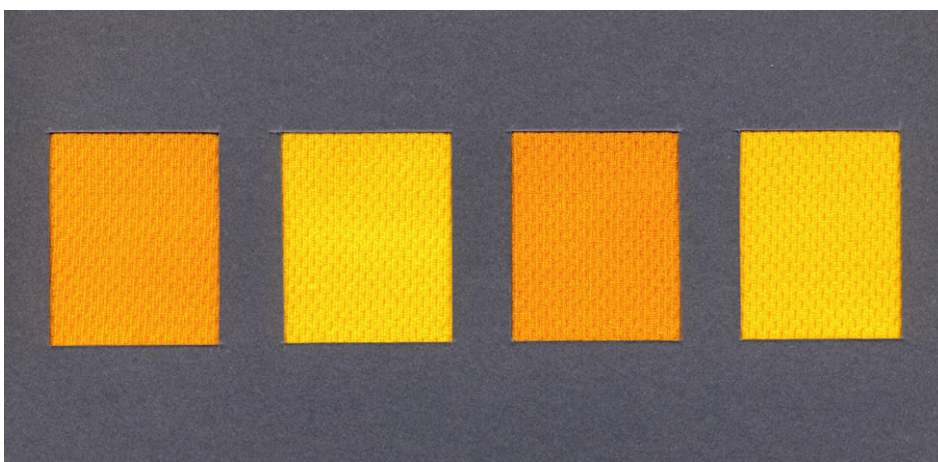
Colour Figure 2. *Dermocybe sanguinea*. (Photograph Mattias Andersson).



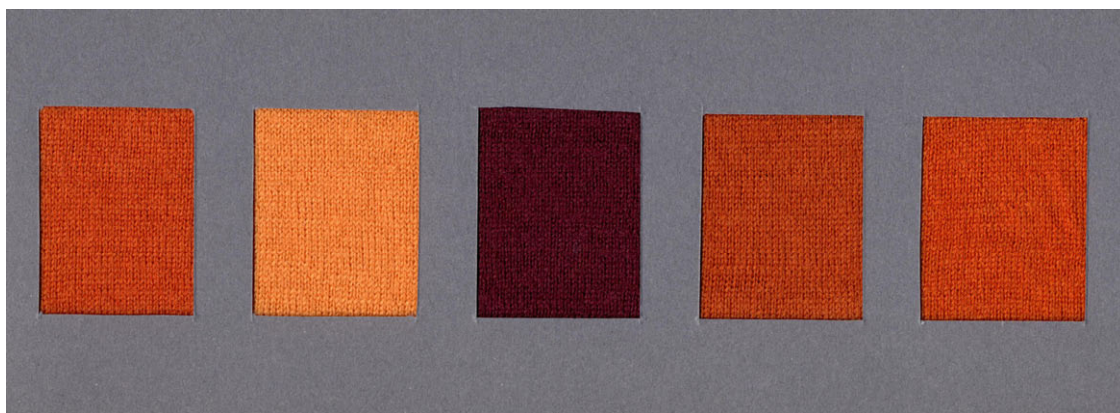
Colour Figure 3. *Dermocybe sanguinea*. (Photograph Mattias Andersson).



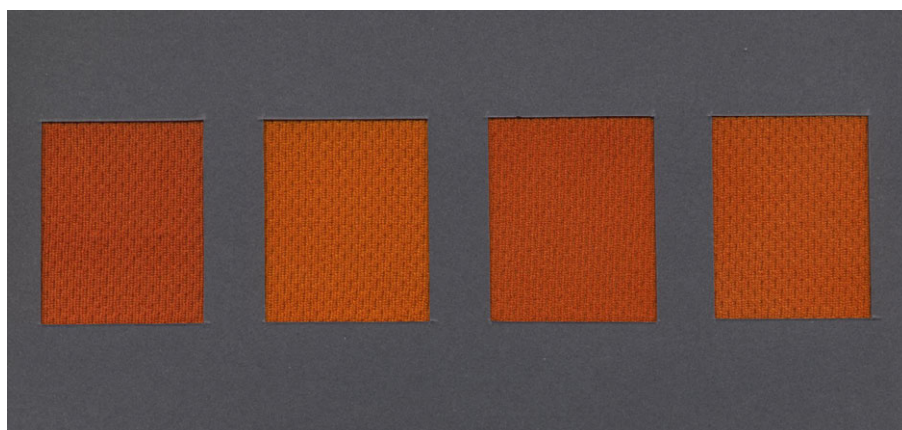
Colour Figure 4a. Wool dyed with emodin in a low pH dyebath. Samples from the left: **1**: no mordant used, **2**: 10% $\text{KAl}(\text{SO}_4)_2$, **3**: 5% $\text{K}_2\text{Cr}_2\text{O}_7$, **4**: 5% CoSO_4 and **5**: 5% FeSO_4 . The numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



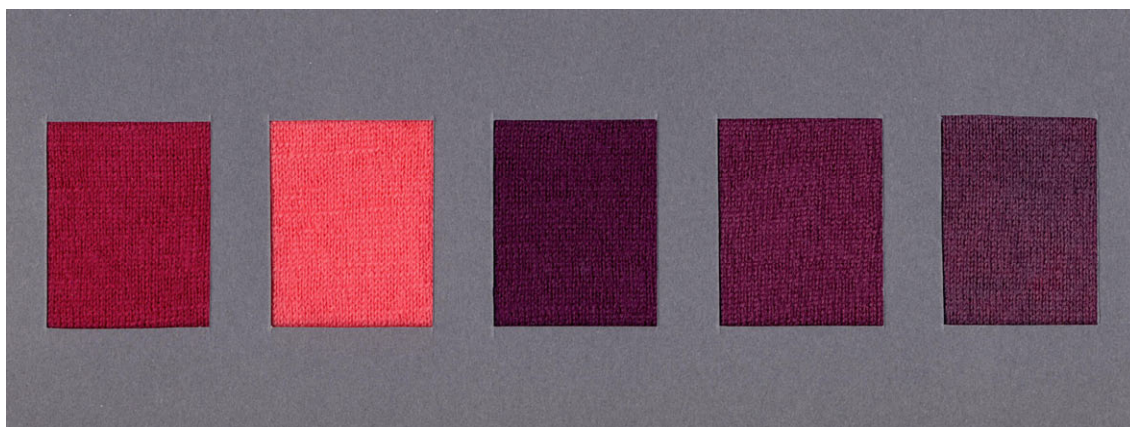
Colour Figure 4b. Polyamide dyed with emodin in a low pH dyebath. Samples from the left: **16**: no mordant used, **17**: 10% $\text{KAl}(\text{SO}_4)_2$, **18**: 5% CoSO_4 and **19**: 5% FeSO_4 . The numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



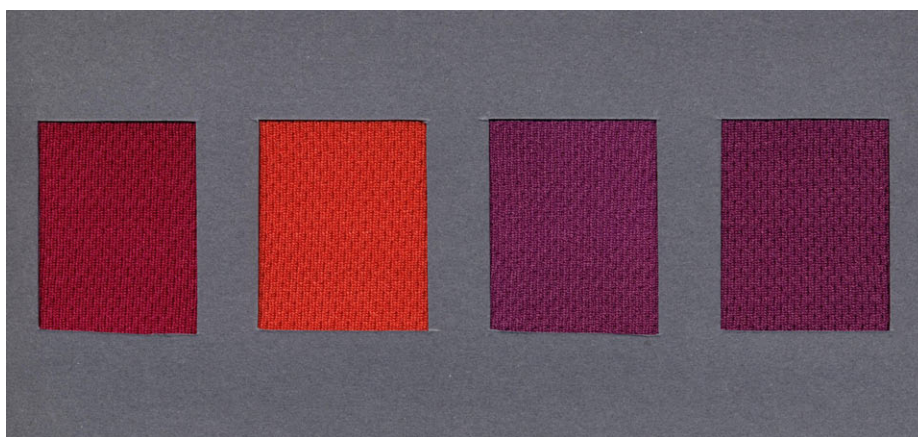
Colour Figure 5a. Wool dyed with emodin in the high pH dyebath. Samples from the left: **6**: no mordant used, **7**: 10% $\text{KAl}(\text{SO}_4)_2$, **8**: 1% $\text{K}_2\text{Cr}_2\text{O}_7$, **9**: 1% CoSO_4 and **10**: 1% FeSO_4 . The numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



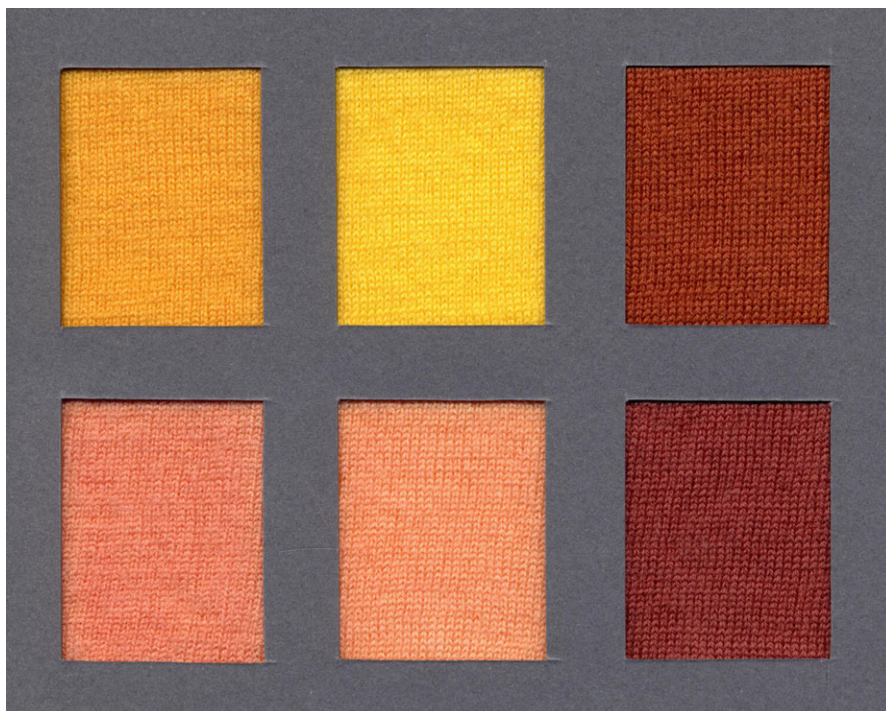
Colour Figure 5b. Polyamide dyed with emodin in the high pH dyebath. Samples from the left: **20**: no mordant used, **21**: 10% $\text{KAl}(\text{SO}_4)_2$, **22**: 1% CoSO_4 and **23**: 1% FeSO_4 . The numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



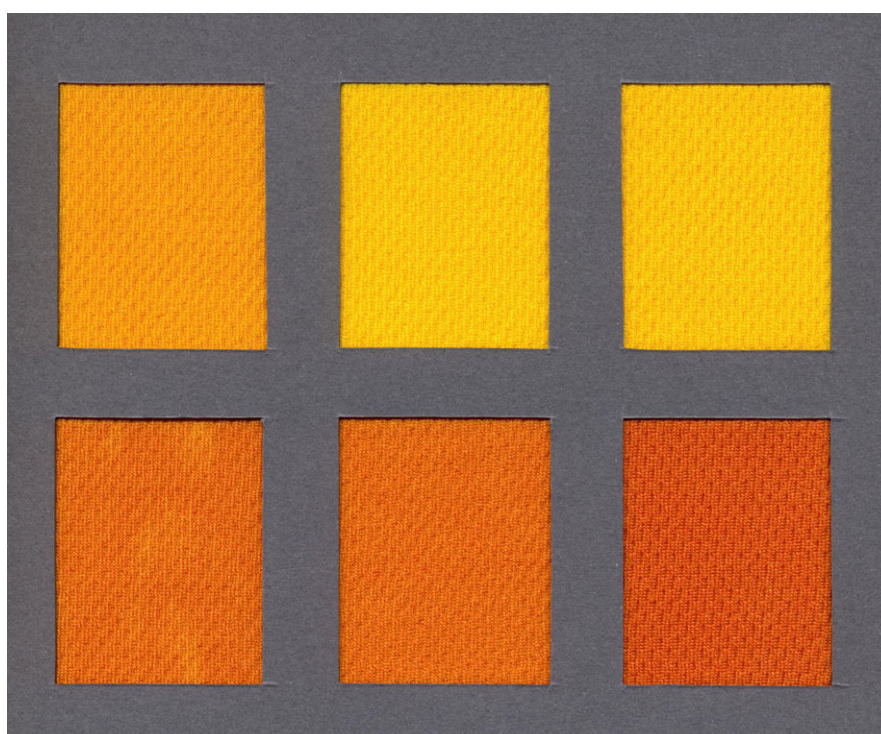
Colour Figure 6a. Wool dyed with dermocybin. Samples from the left: **11**: no mordant used, **12**: 10% (owf) $\text{KAl}(\text{SO}_4)_2$, **13**: 1% $\text{K}_2\text{Cr}_2\text{O}_7$, **14**: 1% CoSO_4 and **15**: 1% FeSO_4 . Numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



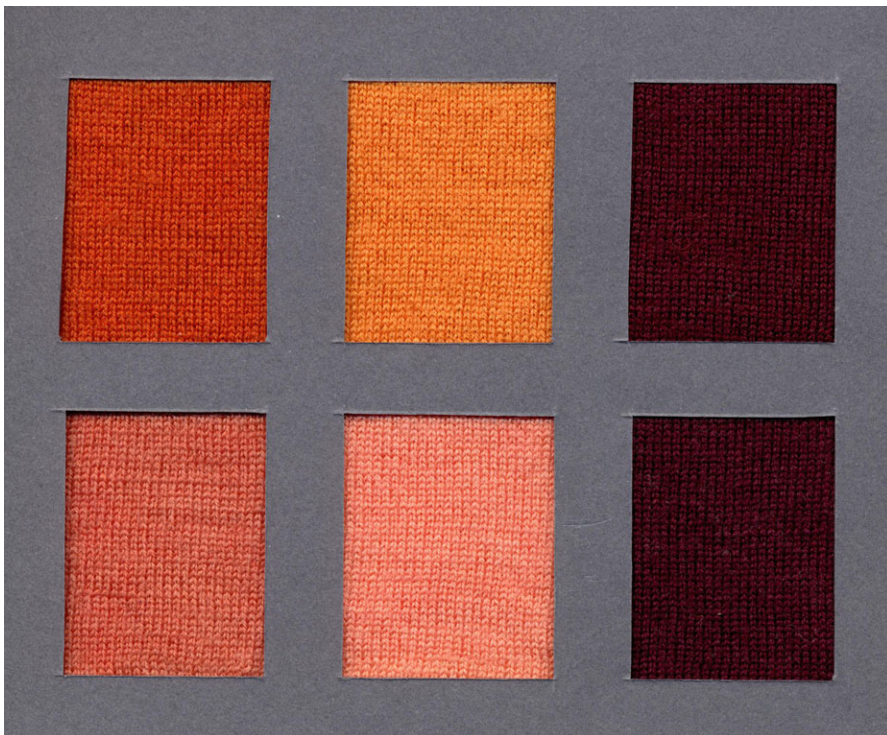
Colour Figure 6b. Polyamide dyed with dermocybin. Samples from the left: **24**: no mordant used, **25**: 10% (owf) $\text{KAl}(\text{SO}_4)_2$, **26**: 1% CoSO_4 and **27**: 1% FeSO_4 . Numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



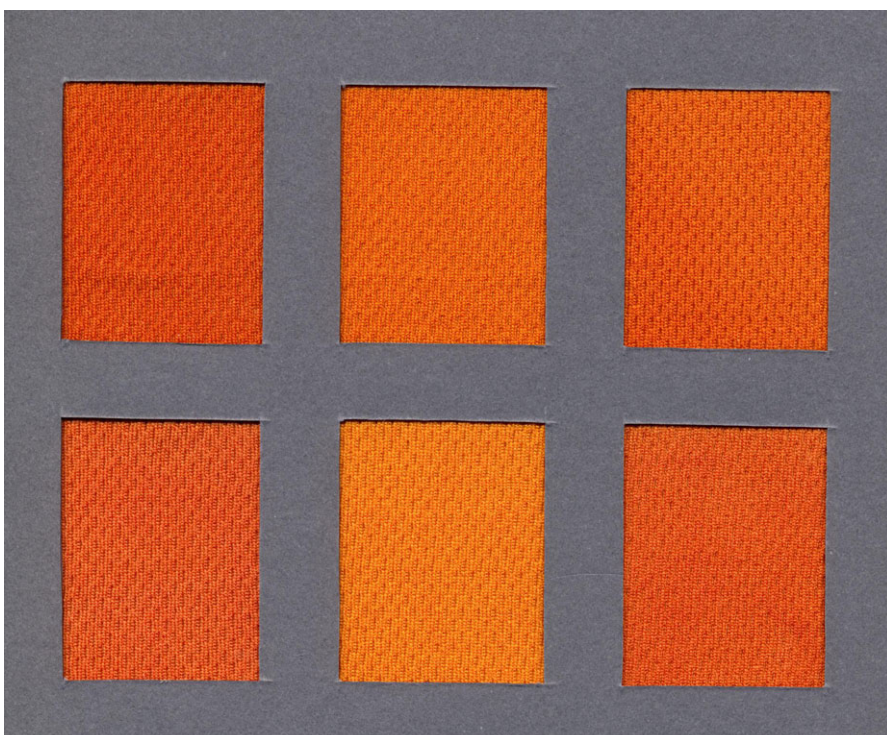
Colour Figure 7. The colour change after washing. Wool samples were dyed with emodin in the low pH dyebath. The top row shows the unwashed dyed samples and the bottom row the same samples after the ISO 105-C06-1994 test. Samples from the left: **1**: no mordant used, **2**: 10% (owf) $\text{KAl}(\text{SO}_4)_2$ and **3**: 5% $\text{K}_2\text{Cr}_2\text{O}_7$. The numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



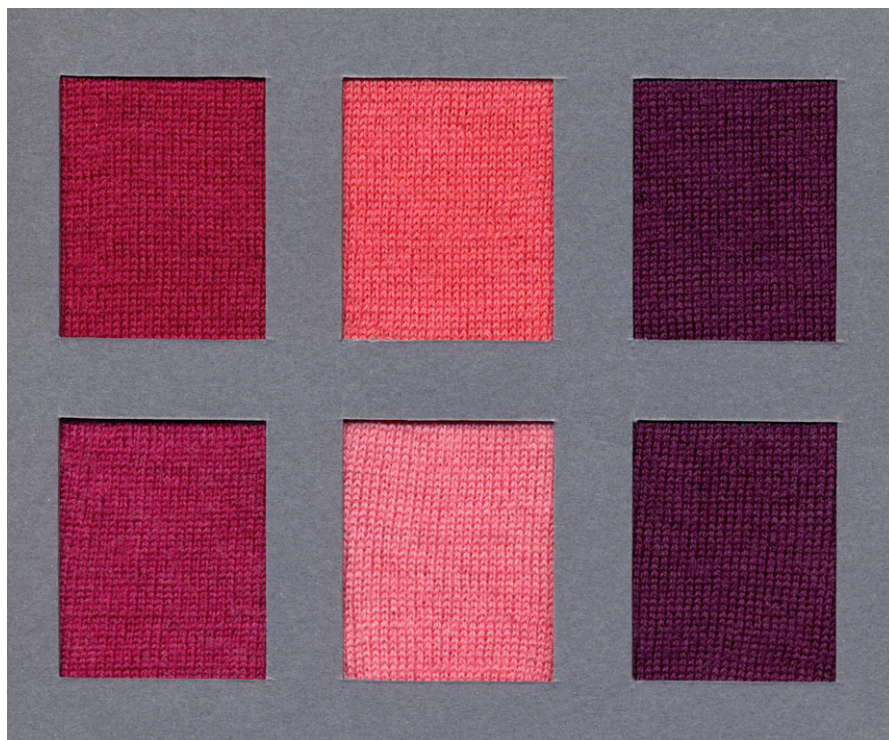
Colour Figure 8. The colour change of the polyamide samples after washing. Samples were dyed with emodin in the low pH dyebath. The top row shows the unwashed dyed samples and the bottom row the same samples after the ISO 105-C06-1994 test. Samples from the left: **16**: no mordant used, **17**: 10% (owf) $\text{KAl}(\text{SO}_4)_2$ and **18**: 5% CoSO_4 . The numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



Colour Figure 9. The colour change after washing. Wool samples were dyed with emodin in the high pH dyebath. The top row shows the unwashed dyed samples and the bottom row the same samples after the ISO 105-C06-1994 test. Samples from the left: **6**: no mordant used, **7**: 10% (owf) $\text{KAl}(\text{SO}_4)_2$ and **8**: 1% $\text{K}_2\text{Cr}_2\text{O}_7$. The numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



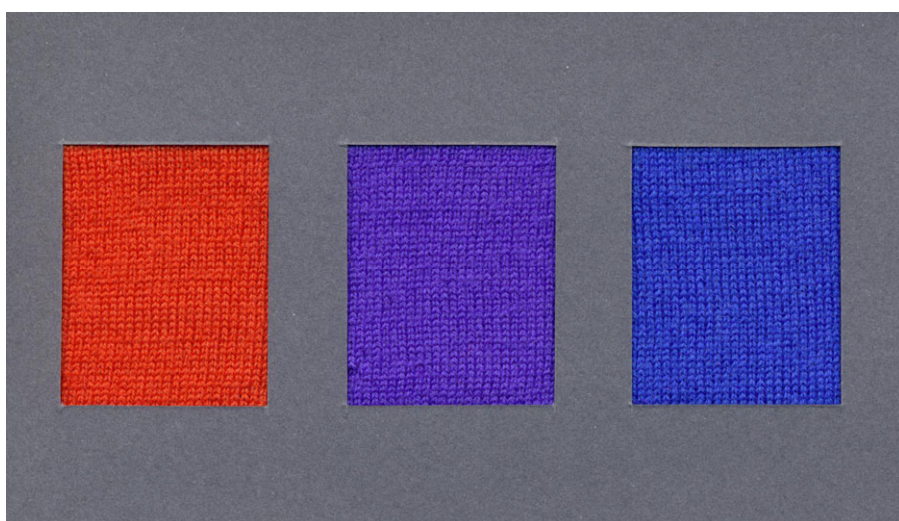
Colour Figure 10. The colour change of the polyamide samples after washing. Samples were dyed with emodin in the high pH dyebath. The top row shows the unwashed dyed samples and the bottom row the same samples after the ISO 105-C06-1994 test. Samples from the left: **20**: no mordant used, **21**: 10% (owf) $\text{KAl}(\text{SO}_4)_2$ and **22**: 1% CoSO_4 . The numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



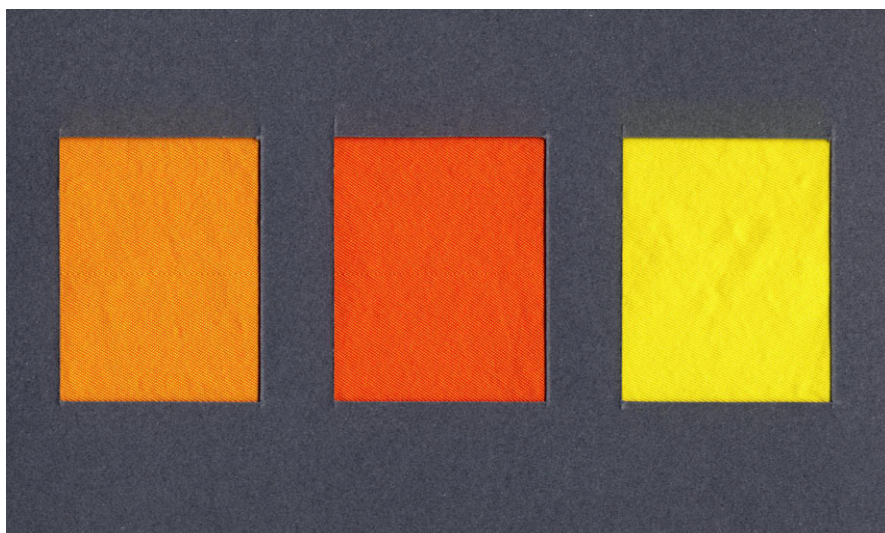
Colour Figure 11. The colour change of dermocybin dyed wool samples after washing. The top row shows the unwashed dyed samples and the bottom row the same samples after the ISO 105-C06-1994 test. Samples from the left: **11**: no mordant used, **12**: 10% (owf) $\text{KAl}(\text{SO}_4)_2$ and **13**: 1% $\text{K}_2\text{Cr}_2\text{O}_7$. Numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



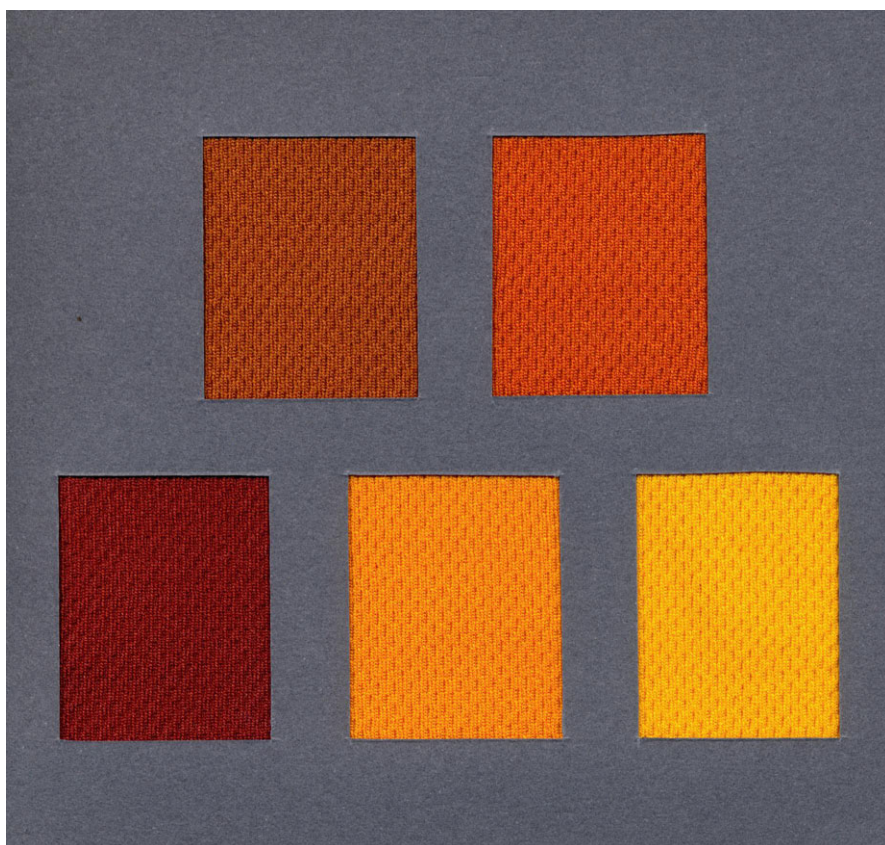
Colour Figure 12. The colour change of the dermocybin dyed polyamide samples after washing. The top row shows the unwashed dyed samples and the bottom row the same samples after the ISO 105-C06-1994 test. Samples from the left: **24**: no mordant used, **25**: 10% (owf) $\text{KAl}(\text{SO}_4)_2$ and **26**: 1% CoSO_4 . Numbers in boldface refer to the dyeing conditions explained in Table I in Publication IV.



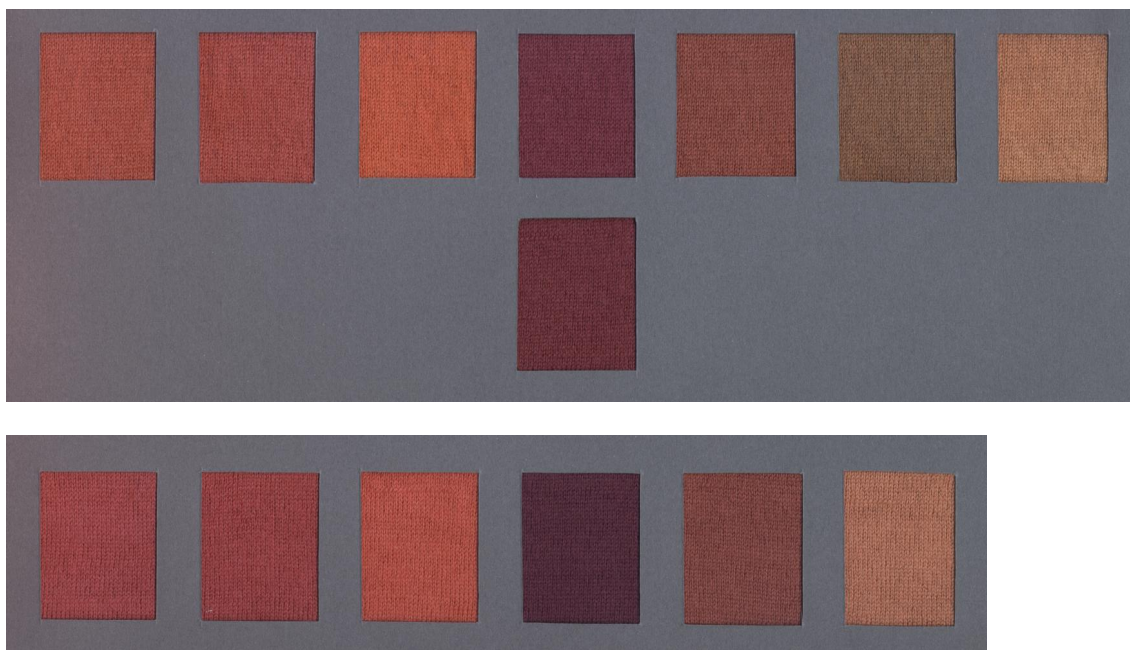
Colour Figure 13. Wool dyed with acid dyes. Samples from the left: the mixture of dermorubin and 5-chlorodermorubin, CI Acid Violet 109 and CI Acid Blue 80, respectively.



Colour Figure 14. Polyester fabrics dyed with Fraction 1, dermocybin and emodin, respectively, using the high-temperature disperse dyeing technique.



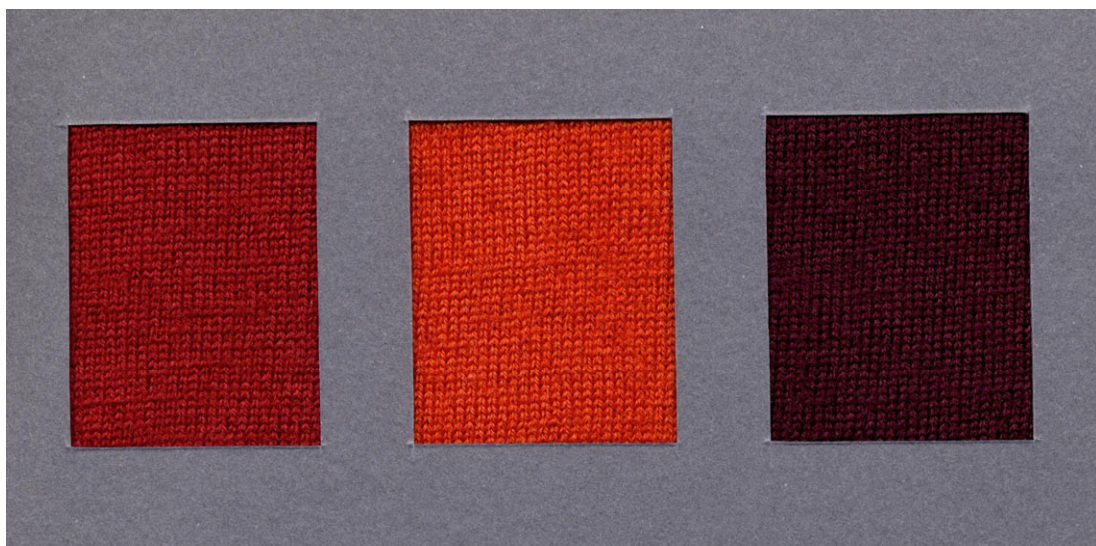
Colour Figure 15. Polyamide fabrics that were dyed using the high-temperature disperse dyeing technique. The top row: Fraction 1 was used as a dye in the first sample. In the second sample Fraction 1 together with 1% (owf) $\text{KAl}(\text{SO}_4)_2$ were used. The bottom row: dermocybin, emodin and emodin together with 1% $\text{KAl}(\text{SO}_4)_2$ were used as dyes, respectively.



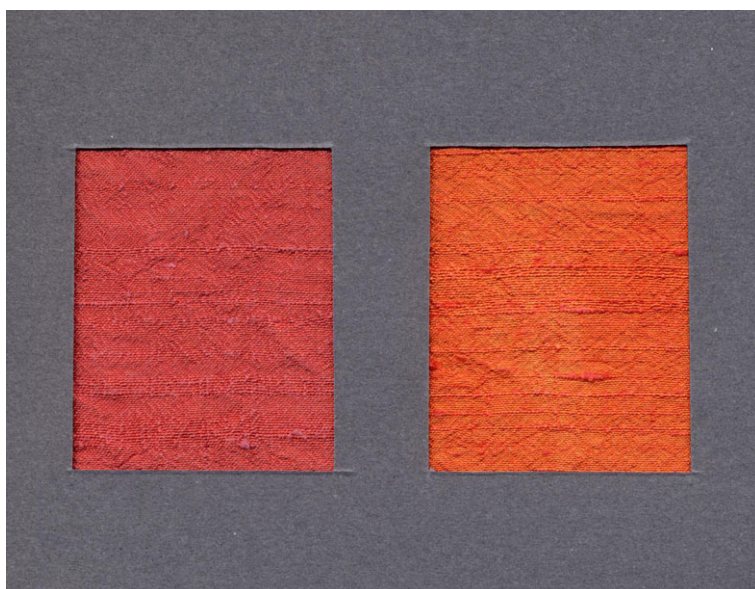
Colour Figure 16. Wool dyed with Fraction 1. In the upper board, 0.01 M Na_2HPO_4 was used as an electrolyte. Samples from the left: **1**: no Glauber's salt and no mordant used, **2**: no mordant, **3**: 1% (owf) $\text{KAl}(\text{SO}_4)_2$, **4a** (top): 1% $\text{K}_2\text{Cr}_2\text{O}_7$, **4b** (bottom): 0.5% $\text{K}_2\text{Cr}_2\text{O}_7$, **5**: 1% CoSO_4 , **6**: 1% FeSO_4 and **7**: 1% CuSO_4 . In the lower board 0.1 M Na_2HPO_4 was used as an electrolyte. Samples from the left: **8**: no Glauber's salt and no mordant used, **9**: no mordant, **10**: 1% (owf) $\text{KAl}(\text{SO}_4)_2$, **11**: 1% $\text{K}_2\text{Cr}_2\text{O}_7$, **12**: 1% CoSO_4 and **13**: 1% CuSO_4 , respectively. The numbers in boldface refer to the dyeing conditions explained in Table IV in the experimental part.



Colour Figure 17. The colour differences between the dyed unwashed (the top row) and dyed washed (the bottom row) wool fabrics. The samples were dyed with Fraction 1 using 0.01 M Na_2HPO_4 as an electrolyte. Samples from the left. **1**: no Glauber's salt and no mordant, **2**: no mordant, **3**: 1% (owf) $\text{KAl}(\text{SO}_4)_2$, **4a**: 1% $\text{K}_2\text{Cr}_2\text{O}_7$, **5**: 1% CoSO_4 , **6**: 1% FeSO_4 and **7**: 1% CuSO_4 . The numbers in boldface refer to the dyeing conditions explained in Table IV in the experimental part.



Colour Figure 18a. Wool fabrics dyed with the anthraquinone aglycones of the crude extract of *Dermocybe sanguinea*. Samples from the left: **14**: no mordant used, **15**: 10% (owf) $\text{KAl}(\text{SO}_4)_2$ and **16**: 1% $\text{K}_2\text{Cr}_2\text{O}_7$. Numbers in boldface refer to the dyeing conditions explained in Table III in the experimental part.



Colour Figure 18b. Silk fabrics dyed with the anthraquinone aglycones of the crude extract of *Dermocybe sanguinea*. Samples from the left: **17**: no mordant used and **18**: 10% (owf) $\text{KAl}(\text{SO}_4)_2$. Numbers in boldface refer to the dyeing conditions explained in Table III in the experimental part.

APPENDIX 3.

Table IV. The uptake of emodin and dermocybin in the mordant dyeing experiments. The numbers in boldface correspond to the dyeing conditions explained in Table I in Publication IV.

	Wool		Polyamide	
	No.	Uptake %	No.	Uptake %
Emodin, pH lower:				
No mordant	1	97	16	98
KAl(SO ₄) ₂	2	70	17	98
K ₂ Cr ₂ O ₇	3	96		
CoSO ₄	4	95	18	100
FeSO ₄	5	97	19	97
Emodin, pH higher:				
No mordant	6	59	20	49
KAl(SO ₄) ₂	7	54	21	89
K ₂ Cr ₂ O ₇	8	85		
CoSO ₄	9	66	22	64
FeSO ₄	10	61	23	72
Dermocybin:				
No mordant	11	87	24	58
KAl(SO ₄) ₂	12	83	25	86
K ₂ Cr ₂ O ₇	13	91		
CoSO ₄	14	96	26	86
FeSO ₄	15	93	27	77

APPENDIX 4.

Table VII. The uptake of Fraction 1 anthraquinones for wool. The numbers in boldface correspond to the dyeing conditions explained in Table III. Gls = Glauber's salt.

	No.	Uptake %
Fraction 1, 0.01 M buffer:		
No Gls, no mordant	1	68
No mordant	2	74
KAl(SO ₄) ₂	3	83
K ₂ Cr ₂ O ₇ (1%)	4a	84
K ₂ Cr ₂ O ₇ (0.5%)	4b	81
CoSO ₄	5	85
FeSO ₄	6	-
CuSO ₄	7	93
Fraction 1, 0.1 M buffer:		
No Gls, no mordant	8	60
No mordant	9	61
KAl(SO ₄) ₂	10	75
K ₂ Cr ₂ O ₇	11	93
CoSO ₄	12	-
CuSO ₄	13	61